

03/16/99  
jc595 U.S. PTO

Patent  
Attorney's Docket No. 017753-113

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

UTILITY PATENT  
APPLICATION TRANSMITTAL LETTER

jc511 U.S. PTO  
09/27/006  
03/16/99

Box PATENT APPLICATION  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Enclosed for filing is the utility patent application of Jean-Pierre ROBIN; Julie BLANCHARD; Sylvie CAVOLEAU; Ludovic CHAUVIAT; Sandra CHARBONNEL; Robert DHAL; Gilles DUJARDIN; Florence FOURNIER; Chrystelle GILET; Laurent GIRODIER; Laurence MEVELEC; Sandrine POUTOT; Sylvie ROUAUD for NOVEL CEPHALOTAXANE DERIVATIVES AND PROCESS FOR THEIR PREPARATION.

Also enclosed are:

- ☒ 2 sheet(s) of ☒ formal ☐ informal drawing(s);
- ☒ a claim for foreign priority under 35 U.S.C. §§ 119 and/or 365 is ☒ hereby made to 98 03492 filed in France on March 30, 1998; ☒ in the declaration;
- ☒ a certified copy of the priority document;
- ☐ a Constructive Petition for Extensions of Time;
- ☒ One statement(s) claiming small entity status;
- ☐ an Assignment document;
- ☐ an Information Disclosure Statement; and
- ☐ Other: \_\_\_\_\_

The declaration of the inventor(s) ☒ also is enclosed ☐ will follow.

- ☐ Please amend the specification by inserting before the first line the sentence --This application claims priority under 35 U.S.C. §§119 and/or 365 to \_\_ filed in \_\_ on \_\_; the entire content of which is hereby incorporated by reference.--

The filing fee has been calculated as follows [ ] and in accordance with the enclosed preliminary amendment:

CLAIMS					
	NO. OF CLAIMS		EXTRA CLAIMS	RATE	FEE
Basic Application Fee					\$760.00
Total Claims	87	MINUS 20 =	67	x \$18.00	1206.00
Independent Claims	26	MINUS 3 =	23	x \$78.00	1794.00
If multiple dependent claims are presented, add \$260.00					
Total Application Fee					3760.00
If verified Statement claiming small entity status is enclosed, subtract 50% of Total Application Fee					1880.00
Add Assignment Recording Fee of \$40.00 if Assignment document is enclosed					40.00
<b>TOTAL APPLICATION FEE DUE</b>					<b>1920.00</b>

☒ A check in the amount of \$ 1920.00 is enclosed for the fee due.

☐ Charge \$ \_\_\_\_\_ to Deposit Account No. 02-4800 for the fee due.

Please address all correspondence concerning the present application to:

Norman H. Stepno  
Burns, Doane, Swecker & Mathis, L.L.P.  
P.O. Box 1404  
Alexandria, Virginia 22313-1404.

The Commissioner is hereby authorized to charge any appropriate fees under 37 C.F.R. §§ 1.16, 1.17 and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800. This paper is submitted in triplicate.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

Date: March 16, 1999

By: \_\_\_\_\_

Teresa Stanek Rea  
Registration No. 30,427

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

Attny's Docket No. \_\_\_\_\_

**SMALL ENTITY DECLARATION**  
[37 CFR 1.9(c-f)]

Each undersigned declares that:

- (1) ☐ the application attached hereto  
(2) ☐ U.S. Application Serial No. \_\_\_\_\_ filed \_\_\_\_\_  
(3) ☐ U.S. Patent No. \_\_\_\_\_ Issued \_\_\_\_\_

is entitled to the benefits of "small entity" status for paying reduced fees under 35 USC 41(a) and (b) to the Patent and Trademark Office by virtue of the following:

(4) ☐ Each undersigned declares that he/she qualifies as an independent inventor, or would qualify had he/she made the invention, as defined in 37 CFR 1.9(c).

(5) ☐ The undersigned declares that he/she is an official empowered to act on behalf of the concern identified below; that this concern qualifies as a small business concern as defined in 37 CFR 1.9(d).

(6) ☐ The undersigned declares the he/she is an official empowered to act on behalf of the organization identified below; that this organization qualifies as a nonprofit organization as defined in

- (a) ☐ 37 CFR 1.9(e)(1)  
(b) ☐ 37 CFR 1.9(e)(2)  
(c) ☐ 37 CFR 1.9(e)(3)  
(d) ☐ 37 CFR 1.9(e)(4)

State law of TEXAS, USA

(7) Each person, concern or organization to which I/we have assigned, granted, conveyed or licensed, or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- (a) ☐ no such person, concern or organization  
(b) ☐ persons, concerns or organizations listed below

[a separate declaration is required from each named person, concern or organization having rights to this invention averring to their status as "small entities."]

Full Name ONCOPHARM CORPORATION

Address 7707 Fannin, Suite 120 - HOUSTON, TEXAS 77054 USA

☐ Individual ☒ Small Business Concern ☐ Nonprofit Organization

I/we acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement of small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I/we hereby declare all statements made herein of his/her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application, any patent issued thereon, or any patent to which this declaration is directed.

(8) \_\_\_\_\_  
Typed Name of Inventor Signature Date

\_\_\_\_\_  
Typed Name of Inventor Signature Date

(9) ONCOPHARM CORPORATION

Name of Small Business Concern or Nonprofit Organization

Jean-Pierre ROBIN By [Signature] Date MARCH 5, 1999  
Typed Name Signature Date

PRESIDENT AND CEO  
Title of Signatory

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of )  
 )  
Jean-Pierre ROBIN et al ) Group Art Unit: Unassigned  
 )  
Application No.: Unassigned) Examiner: Unassigned  
 )  
Filing Date: March 16, 1999 )  
 )  
For: NOVEL CEPHALOTAXANE )  
 )  
DERIVATIVES AND PROCESS )  
 )  
FOR THEIR PREPARATION )

**PRELIMINARY AMENDMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-captioned application as follows:

**IN THE CLAIMS:**

Please amend the claims as follows:

1. (Amended) A process for the preparation of sidechain-bearing cephalotaxane of the following formula and/or a salt thereof



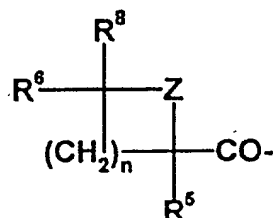
where

$\Omega$  ("omega") is a representative radical of the chain terminal moiety and -CO- is the carbonyl of the ester group bonded to cephalotaxane;

the  $\Omega$ -CO-radical is corresponding:

- either to the following substituted heterocycloalkane formula;

09270006-034599



where n [is included between] ranges from 0 [and] to 8;

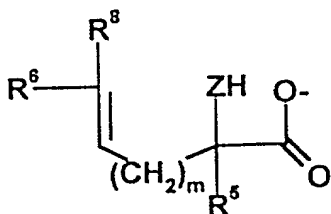
Z is oxygen, nitrogen or sulfur heteroatom;

R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are independently hydrogen;

hydrocarbon radical, saturated, [insaturated] unsaturated or aromatic, linear or [ramified] branched and/or cyclic, [especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl, of said radical] optionally including [or not] heteroatom(s); R<sup>6</sup> and R<sup>8</sup> may be [included] present in a cycle;

oxygen ether bearing one of the former radicals;

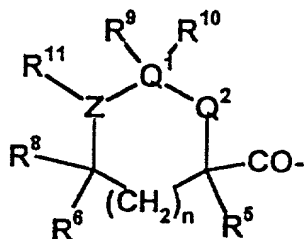
- or to the following linear alkene formula;



where m [is included between] ranges from 1 [and] to 8;

R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined above;

- or to the following formula;



where n, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined above;

Z and Q<sup>2</sup> are independently oxygen, nitrogen or sulfur heteroatom;

Q<sup>1</sup> is carbon, silicium or phosphorus atom;

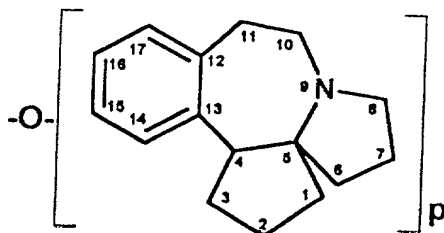
R<sup>9</sup> and R<sup>10</sup> are independently hydrogen, alkoxy, hydrocarbon radical, optionally including [or not] heteroatom(s), saturated, unsaturated or aromatic, linear or [ramified] branched and/or cyclic [,especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl];

R<sup>9</sup> and/or R<sup>10</sup> having the ability to be null or taken together to make an heteroatom and/or make a multiple bond with Q<sup>1</sup>, R<sup>9</sup> and R<sup>11</sup> having the ability to be null to make a multiple bond between the two atoms of carbon bearing them; and

R<sup>11</sup> is hydrogen, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl or alkylcarbonyl;

where

-O-CTX is cephalotaxine moiety of the following formula or a salt thereof;



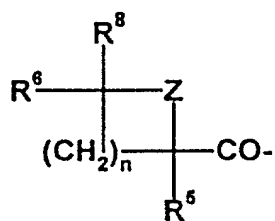
where p is equal to 1 or 2;

the two types of radicals -Ω and -CTX above-mentioned being bonded with an ester bond -CO-O-

the said process bringing together:

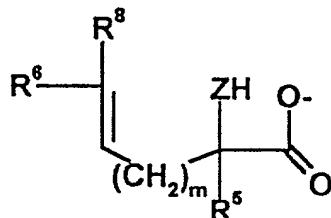
- either carboxylic acid with general formula Ω-CO-OH or a salt thereof;
- or an activated form of an acid with general formula Ω-CO-A or a salt thereof,

with Ω-CO of the following formula:



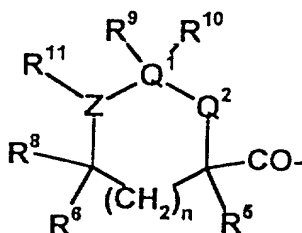
where n, Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined above;

where  $\Omega$ -CO of the following formula:



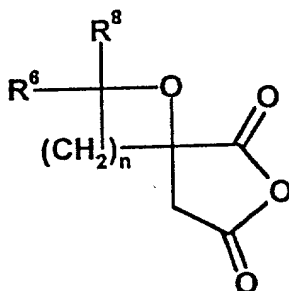
m [is included between] ranges from 1 [and] to 8, Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined above;

where  $\Omega$ -CO of the following formula:



where n, Z, Q<sup>1</sup>, Q<sup>2</sup>, R<sup>5</sup>, R<sup>6</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup> and R<sup>11</sup> are as defined above A represents:

- either cyclic anhydride of the following formula:



where n, R<sup>6</sup> and R<sup>8</sup> are as defined above;



this reaction has been completed by methylation of the primary carboxyl thus formed, with:

- either a hydroxyl group bearing cephalotaxane or a salt thereof of the formula H-O-CTX, where CTX are as defined above;
- or a metallic alcoxide of the formula M-O-CTX, where CTX are as defined above and M is a metal;
- or an activated form of its hydroxyl group of the formula Y-O-CTX, where -O-CTX is as defined above and Y is, either a leaving group to allow a negative charge on oxygen atom by cleavage between Y- and -O-CTX, or to allow a carbocation by cleavage between Y-O- and -CTX;

with the possible presence of one or several reaction additives to form said sidechain-bearing cephalotaxane and/or a salt thereof.

Claim 4, line 1, delete "anyone of claims 1 to 3" and insert --claim 1--;

Claim 5, line 1, delete "anyone of claims 1 to 3" and insert --claim 1--;

Claim 6, line 1, delete "anyone of claims 1 to 5" and insert --claim 1--;

Claim 7, line 1, delete "anyone of claims 1 to 5" and insert --claim 1--;

Claim 9, line 1, delete "anyone of claims 1 to 8" and insert --claim 1--;

Claim 9, line 2, delete "where  $\Omega$  is as defined according to claim 1";

Claim 10, line 1, delete "anyone of claims 1 to 8" and insert --claim 1--;

Claim 11, line 1, delete "anyone of claims 1 to 8" and insert --claim 1--;

Claim 12, line 1, delete "anyone of claims 1 to 8" and insert --claim 1--;

Claim 13, line 1, delete "anyone of claims 1 to 8" and insert --claim 1--;

Claim 15, line 1, delete "anyone of claims 1 to 8" and insert --claim 1--;

Claim 16, line 2, delete "as defined according to claim 1";

Claim 18, line 1, delete "previous claims" and insert --claim 1--;

Claim 18, lines 2-3, delete "such as tertiary amine for example";

Claim 20, line 1, delete "anyone of previous claims" and insert --claim 1--;

Claim 20, line 2, delete "is corresponding" and insert --corresponds--;

Claim 20, delete line 4;

Claim 20, delete line 7;

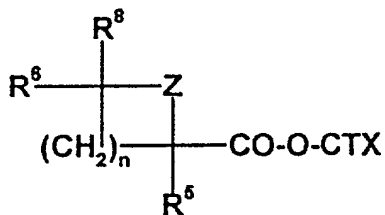
Claim 20, line 8, delete "himself";

21. (Amended) The process according to [anyone of claims 1 to 20] claim 1,  
wherein M is an alkaline metal [such as lithium, potassium or sodium].

Claim 22, line 1, delete "is";

Claim 23, line 1, delete "is";

24. (Amended) A sidechain-bearing cephalotaxane corresponding to the following  
formula and/or a salt thereof:



where

n [is included between] ranges from 9 [and] to 8;

Z is oxygen, nitrogen or sulfur heteroatom;

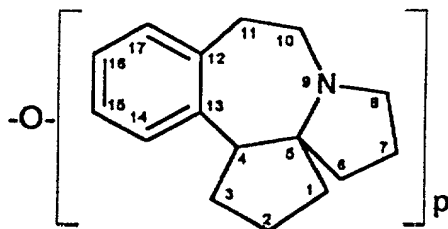
R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are independently hydrogen;

hydrocarbon radical, saturated, [insaturated] unsaturated or aromatic, linear or [ramified] branched and/or cyclic, [especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl, or said radical] optionally including [or not] heteroatom(s);

oxygen ether bearing one of the former radicals;

[CTX is as defined according to anyone of claims 1 to 3];

-O-CTX is cephalotaxine moiety of the following formula or a salt thereof:



where p is equal to 1 or 2;

the two types of radicals -Ω and -CTX above-mentioned being bonded with an ester bond -CO-O-

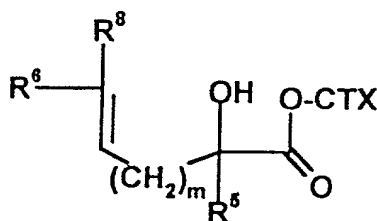
except for compounds where Z is oxygen atom and,

1°) n = 2 or 3, and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OMe or hydroxyl,

1° n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OMe or hydroxyl;

3° n = 3 and simultaneously R<sup>6</sup> is hydroxyl, when R<sup>8</sup> is [methyl] methyl and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical.

25. (Amended) A sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:



where

m, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1, and CTX is as defined according to [anyone of claims 1 to 3] claim 1,

except compound where m = 2, R<sup>5</sup> = CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>, R<sup>6</sup> = R<sup>8</sup> = methyl and [CTX is as defined according to claim 3] -o-CTX is cephalotaxine, where R<sup>1</sup> is hydroxyl, R<sup>2</sup> is methoxyl, R<sup>3</sup> and R<sup>4</sup> are hydrogen.

Claim 27, line 5, delete "as";

Claim 27, lines 5-6, delete "anyone of claims 1 to 3" and insert --claim 1--;

Claim 34, line 1, delete "anyone of claims 1 to 21" and insert --claim 1--;

Claim 34, line 29, after "the said" insert --optionally--;

Claim 34, line 29, delete "or not";

Claim 34, line 30, delete "insaturated" and insert --unsaturated--;

Claim 34, line 30, delete "ramified" and insert --branched--;

Claim 34, lines 31-32, delete "especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, or heterocycloalkyl";

Claim 35, line 6, delete "preferably dichloromethane,";

Claim 35, line 10, delete "where n, CTX, R<sup>5</sup>, R<sup>6</sup> et R<sup>8</sup> are defined according to claim 1";

Claim 36, line 1, delete "anyone of claims 1 to 21 and claim 34" and insert --claim 1--;

Claim 36, delete line 4;

Claim 36, line 6, delete "such as" and insert --wherein--;

Claim 36, line 9, delete "of";

Claim 36, line 9, delete "its" and insert --the--;

Claim 36, line 9, delete "form" and insert --forms--;

Claim 37, line 4, delete "where n, Z, R<sup>6</sup>, R<sup>8</sup>, and R<sup>5</sup> are as defined according to claim 1";

Claim 38, line 4, delete "where n, Z, R<sup>6</sup>, R<sup>8</sup>, and R<sup>5</sup> are as defined according to claim 1";

Claim 39, lines 4-5, delete "where n, R<sup>5</sup>, R<sup>6</sup>, R<sup>8</sup>, Z, Q<sup>2</sup>, Q<sup>1</sup>, R<sup>9</sup>, R<sup>10</sup> and R<sup>11</sup> are as defined according to claim 1".

Claim 40, line 1, delete "anyone of claims 36 to 39";

Claim 40, line 3, delete "such as  $\Omega$  and  $\Delta^*$  are as defined according to claim 36";

Claim 40, line 3, delete "the";

Claim 40, lines 5-6, delete "such as  $\Delta^*$  is as defined according to claim 36,  
according the process of the claim 1".

Claim 41, line 1, delete "anyone of claims 36 to 39" and insert --claim 36--;

Claim 41, lines 3-4, delete "such as  $\Omega$  and  $\Delta^*$  are as defined according to claim  
36";

Claim 41, line 4, delete "the";

Claim 41, lines 6-7, delete "such as  $\Delta^*$  is as defined according to claim 36,  
according the process of the claim 1".

Claim 42, line 1, delete "anyone of claims 36 to 39" and insert --claim 36--;

Claim 42, line 3, delete "such as  $\Omega$  and  $\Delta^*$  are as defined according to claim 36";

Claim 42, line 3, delete "the";

Claim 42, lines 5-6, delete "such as  $\Delta^*$  is as defined according to claim 36,  
according the process of the claim 1".

Claim 43, line 1, delete "anyone of claims 36 to 39" and insert --claim 36--;

Claim 43, line 2, delete "just";

Claim 43, line 2, delete "of" and insert --an--;

Claim 43, line 3, delete "the either one or the other of the three" and insert --one of  
the--;

Claim 43, delete line 8;

Claim 44, line 1, delete "anyone of claims 36 to 39" and insert --claim 36--;

Claim 44, line 2, delete "labil" and insert --labile--;

Claim 45, line 1, delete "anyone of claims 36 to 39" and inset --claim 36--;

Claim 45, line 2, delete "labil" and insert --labile--;

Claim 54, line 1, delete "anyone of claims 1 to 21 and 34 to 49" and insert --claim 1--;

Claim 54, line 9, delete the period and inset -- ; --;

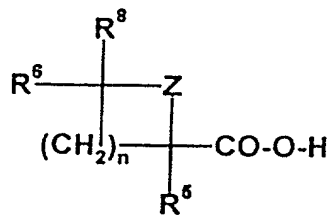
Claim 54, line 15, delete "is included between 1 and 8" and insert --range from t to 8--;

Claim 54, line 19, delete "more generally by the method of the start of art" and inset --by a method--;

Claim 55, line 2, delete "just take place" and insert --is conducted--;

Claim 58, line 1, delete "anyone of claims 54 to 57" and insert --claim 54--;

59. (Amended) The tertiary heterocycloalcanecarboxylic acid, [included its salts and] or salt thereof or each one of its ure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



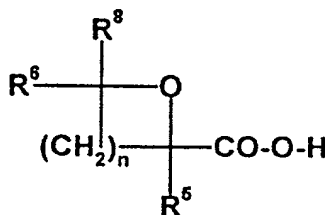
where n [is included between] ranges from 1 [and] to 8, [Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1] Z is oxygen, nitrogen or sulfur heteroatom, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are

independently hydrogen, hydrocarbon radical, saturated, unsaturated or linear or branched and/or cyclic, optionally including heteroatoms, and R<sup>5</sup> is not hydrogen;

except for compounds where Z is oxygen atom and,

- 1°) n = 0 and R<sup>5</sup> is not -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical;  
2°) n = 0 and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical, and R<sup>6</sup> = R<sup>8</sup> = methyl or -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical;  
3°) n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl, and R<sup>5</sup> = OMe or hydroxyl ;  
4°) n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl, and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical or methyl;  
5°) n = 3 and simultaneously R<sup>6</sup> is hydroxyl, and R<sup>8</sup> is methyl, and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical;  
6°) n = 3 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OH or methyl or ethyl.

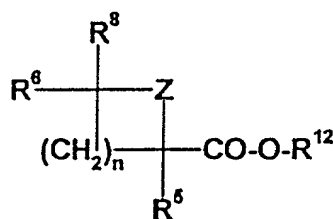
60. (Amended) The tertiary oxacycloalcanecarboxylic acid [included its salts and] or salt thereof or each one of its pure enantiomeric forms of [in] the racemic mixture of [in] the variable composition, according to claim 59 corresponding to the following formula:





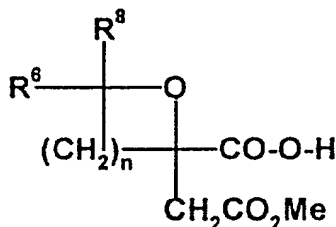
where n [is included between] ranges from 0 [and] to 8, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 59, but are not hydrogen simultaneously.

61. (Amended) The tertiary heterocycloalkane carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture of [in] the variable composition according to claim 54, corresponding to the following formula:



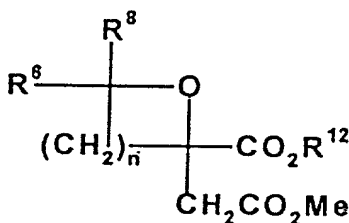
[where n is included between 0 and 8, Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 59,] and R<sup>5</sup> is not hydrogen [, and R<sup>12</sup> is defined according to claim 54].

62. (Amended) The tertiary oxacycloalkane carboxylic hemiester, [included its salts and] or salt thereof or eachone of its pure enantiomeric forms of [in] the racemic mixture or [in] the variable composition according to claim 59, corresponding to the following formula:



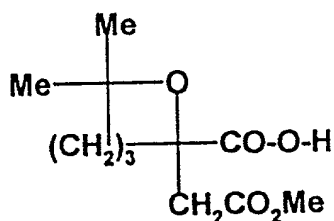
[where n is included between 0 and 8, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 59].

63. (Amended) The tertiary oxacycloalcane carboxylic hemiester, [included its salts and] or salt thereof or each one of its pure enantiomeric forms of [in] the racemix mixture of [in] the variable composition according to claim 54, corresponding to the following formula:

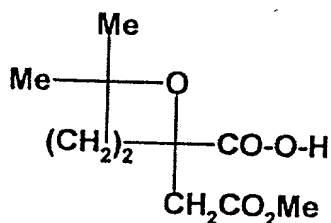


[where n is included between 0 and 8, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 59, R<sup>12</sup> is defined according to claim 54].

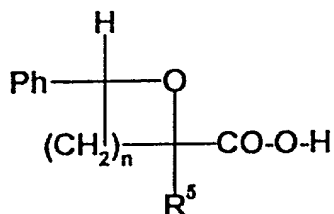
64. (Amended) The tertiary oxacycloalcane carboxylic hemiester or anhydrohomoharringtonic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture of [in] the variable composition, corresponding to the following formula:



65. (Amended) The tertiary oxacycloalkane carboxylic hemiester or anhydro-harringtonic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemix mixture or [in] the variable composition, according to claim 59 corresponding to the following formula:

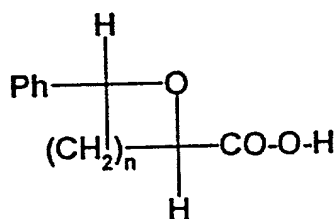


66. (Amended) The tertiary oxacycloalkane carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



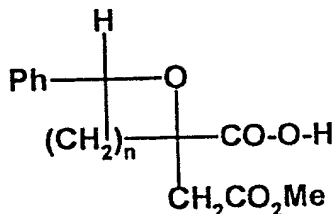
[where n is included between 0 and 8, R<sup>5</sup> is as defined according to claim 59].

67. (Amended) The tertiary oxacycloalcane carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



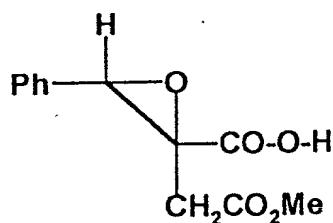
where n [is included between] ranges from 1 [and] to 8.

68. (Amended) The tertiary oxacycloalcane carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:

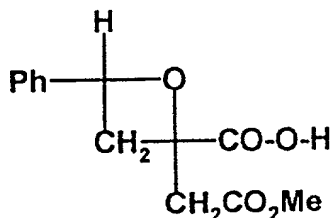


where n [is included between] ranges from 0 [and 8] to 8.

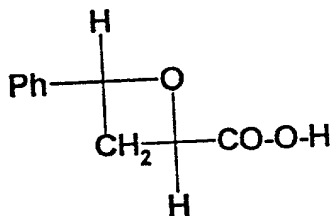
69. (Amended) The tertiary oxacycloalcane carboxylic acid or oxanhydroneoharringtonic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



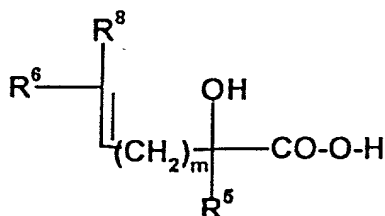
70. (Amended) The tertiary oxacycloalcane carboxylic acid or oxanhydroneohomoharringtonic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



71. (Amended) The tertiary oxacycloalcane carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



72. (Amended) the tertiary alkene carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:

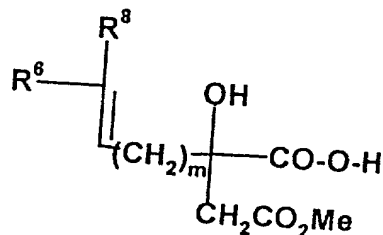


where m [is included between] ranges from 1 [and] to 8, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1, but are not hydrogen simultaneously, and R<sup>5</sup> is not hydrogen or heteroatom.

Claim 73, line 2, after "composition," insert --according to claim 54--;

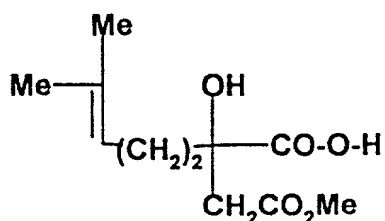
Claim 73, delete lines 5, 6, and 7.

74. (Amended) The tertiary alkene carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, according to claim 59 corresponding to the following formula:

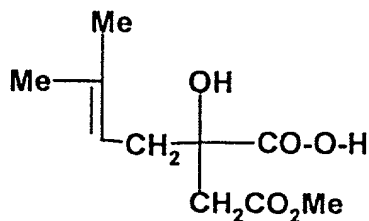


[where m is included between 1 and 8,] wherein R<sup>6</sup> and R<sup>8</sup> [are as defined according to claim 1 but] are not hydrogen.

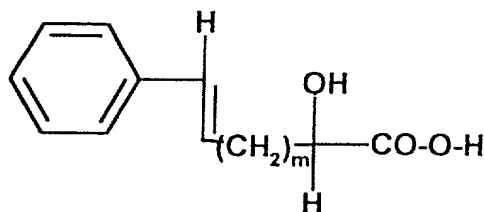
75. (Amended) The tertiary alkene carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture or [in] the variable composition, corresponding to the following formula:



76. (Amended) The tertiary alkene carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the racemic mixture of [in] the variable composition, corresponding to the following formula:



77. (Amended) The tertiary alkene carboxylic acid, [included its salts and] or salt thereof or each one of its pure enantiomeric forms or [in] the variable composition, corresponding to the following formula:



where m [is included between] ranges from 1 to 8.

79. (Amended) The anhydrides of acid according to [anyone of claims 58 to 70] claim 58, of the [general] formula  $\Omega$ -CO-O-CO- $\Omega$  where  $\Omega$  is [as defined according to claim 1] a representative radical of the chain terminal moiety.

80. (Amended) The mixed anhydrides of acid according to [anyone of claims 58 to 70] claim 58, of the [general] formula  $\Omega$ -CO-A where A is [as defined according to anyone of claims 12, 13 or 15] selected from substituents:



methoxyformyloxy of formula MeOCOO-,

trifluoroacetyloxy of formula CF<sub>3</sub>COO-,

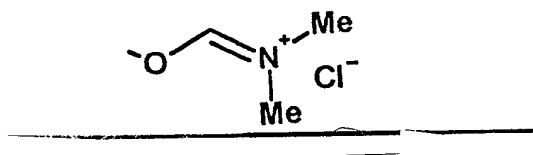
alkylsulfonyloxy of formula RSO<sub>3</sub>-,

phosphoxy of formula (RO)<sub>2</sub>PO-,

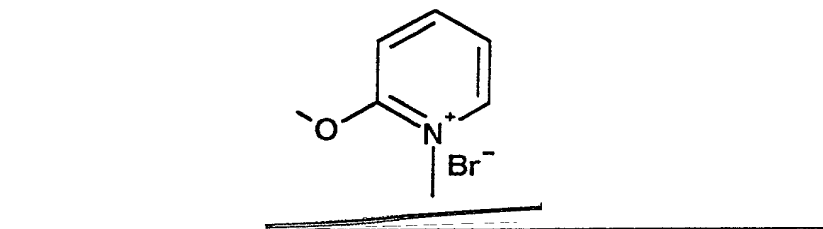
halophosphoxy of formula ROP(Cl)O-,

trialkylsilyloxy of formula R<sub>3</sub>SiO-,

dimethyl-formamidinium chloride of formula:



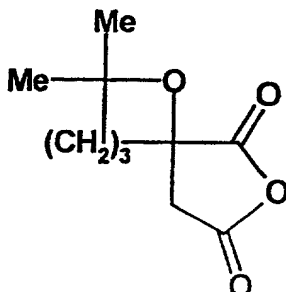
or acyloxy-pyridinium bromide of formula:



Claim 82, line 1, delete "anyone of claims 58 to 70" and insert "--claim 58--";

Claim 82, line 2, delete "general".

83. (Amended) The cyclic anhydrides corresponding to the following formula:



where [n, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1] n ranges from 0 to 8, R<sup>6</sup> and R<sup>8</sup> are independently hydrogen, hydrocarbon radical, saturated, unsaturated or aromatic, linear or branched and/or cyclic optionally including heteroatoms.

Claim 85, line 1, delete "anyone of claims 1 to 21 and 34 to 49" and insert --claim 1--;

Claim 86, line 1, delete "anyone of claims 1 to 21 and 34 to 49" and insert --claim 1--;

Claim 86, line 3, delete "as a pharmaceutical use";

Claim 86, line 5, delete "named";

Claim 86, line 19, delete "especially making use of" and insert --using--;

Claim 86, lines 21-22, delete "preferably n-octadecylsilane,].

**REMARKS**

Entry of the foregoing amendments is respectfully requested.

The claims have been amended to eliminate multiple dependency and to place them in better condition for U.S. patent practice.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: \_\_\_\_\_

Teresa Stanek Rea  
Registration No. 30,427

P.O. Box 1404  
Alexandria, Virginia 22313-1404  
(703) 836-6620

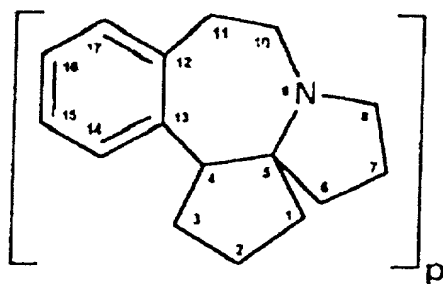
Date: March 16, 1999

09270006 031599  
6597E0 90002260

## Novel cephalotaxane derivatives and process for their preparation

The present invention relates to a process for preparing cephalotaxane  
5 derivatives bearing a side chain.

The term "cephalotaxanes" refers to compounds or salts thereof which have  
a basic skeleton of formula



10

where p is equal to 1 or 2 (it being possible for the two units to be identical or  
different and linked via a single bond or an oxygen atom), which can contain  
various oxygenated substituents (aliphatic or aromatic ethers, free or  
esterified alcohols, substituted or free enols and/or phenols, bridged ethers,  
15 and more generally any substituent usually encountered in the natural state  
on compounds of this type).

Harringtonines are alkaloids which are of high interest in anticancer  
chemotherapy, in particular on certain haematosarcomas which are multi-  
20 resistant to the existing therapies. The selectivity of harringtonines, which is  
based on a novel mechanism of action relating to protein synthesis, is such  
that this series is favoured with a great future in anticancer therapy.

Several literature compilations give a seemingly exhaustive review of all of  
25 the knowledge relating to cephalotaxanes, these compilations being,  
chronologically: [C.R. Smith, Jr, R.G. Powell and K.L. Mikolajczack, Cancer  
Treat. Rep., Vol. 60, 1157 (1976); C.R. Smith, Jr, L. Kenneth,  
K.L. Mikolajczack and R.G. Powell in "Anticancer Agent Based on Natural

Product Model", 391 (1980); Liang Huang and Zhi Xue in "The Alkaloids", Vol. XXIII (A. Brossi Ed.), 157 (1984); M. Suffness and G.A. Cordell in "The Alkaloids, Chemistry and Pharmacology" (A. Brossi Ed.), Vol. 25, 57-69, 295-298 (1987); P.J. O'Dwyer, S.A. King, D.F. Hoth, M. Suffness and B. Leyland-Jones, Journal of Clinical Oncology, 1563 (1986); T. Hudlicky, L.D. Kwart and J.W. Reed, in "Alkaloid: Chemical and Biological Perspectives" (S.W. Pelletier Ed.), Vol. 5, 639 (1987); M.A. Miah, T. Hudlicky and J. Reed in "The Alkaloids", Vol. 51, 199 (1998)].

Antiparasitic activities, in particular on the haematozoon of malaria, have also been recognized [J.M. Whaun and N.D. Brown, Ann. Trop. Med. Par., Vol. 84, 229 (1990)].

Homo-harringtonine (HHT), the most active member of the series, is active at and above daily doses of 2.5 mg/m<sup>2</sup> of body area per 24 hours, i.e., as a guide, at doses twenty times lower than that for Taxol<sup>®</sup>. HHT has already undergone fourteen phase I and II clinical trials and it is the only known product capable of a 70% reinduction of full haematological remissions in patients suffering from chronic myeloid leukaemias that have become resistant to alpha-interferon [S. O'Brien, H. Kantarjian, M. Keating, M. Beran, C. Koler, L.E. Robertson, J. Hester, M. Rios, M. Andreeff and M. Talpaz, Blood, 332 (1995); Leukemia Insights, Vol. 3, No. 1 (1998)].

Harringtonines were extracted over 35 years ago from an exclusively Asiatic cephalotaxacea known as *Cephalotaxus harringtonia*, following the programme of research into novel anticancer agents in the plant kingdom developed by the National Cancer Institute. In fact, the *Cephalotaxus* alkaloids consist essentially (at least 50%) of cephalotaxine, a biosynthetic precursor of the harringtonines, the latter individually representing only a few per cent of the total alkaloids.

Besides their low concentration in the natural state in plant starting material, harringtonines are mixed with many congeners which have very similar chemical structures. Thus, in a high resolution high performance liquid

chromatography (HPLC) chromatogram of a semi-purified alkaloid extract, no less than several tens of cephalotaxine esters are counted.

If we consider that:

5

- on the one hand, harringtonines are generally relatively non-crystallogenic, as is suggested by the flexibility of their side chains, which are generally branched and aliphatic,

10 - on the other hand, these esters, in particular harringtonine and homo-harringtonine, are contaminated with congeners which are themselves biologically active and very difficult to separate out, even by high resolution analytical HPLC,

15 the current state of the art does not allow these compounds to be produced viably on the industrial scale as regards the purity required for pharmaceutical active principles.

Although biosynthetically similar to the alkaloids of the genus *Erythrina*,  
20 cephalotaxanes are alkaloids which have a unique structure in nature, encountered only in the genus *Cephalotaxus*, which is the only genus of the Cephalotaxacea family. On the other hand, the side chains of the various harringtonine congeners are all derived from the methyl hemiester of the primary carboxyl of (2R) citramalic acid **3a** (see Scheme 1 attached) by  
25 substitution of the tertiary methyl using alkyl or aralkyl radicals which may themselves be unsubstituted or substituted with tertiary hydroxyls, it then being possible for the latter to form a cyclic ether with a tertiary alcohol (anhydro derivatives).

30 The attached Scheme 1 shows the main examples of harringtonine congeners, which all have significant cytostatic activity to different degrees. None of the artificial analogous cephalotaxine esters synthesized hitherto in the literature has at least the sub-structure **3b** (see Scheme 1) and lack significant cytostatic activity.

It is worthwhile pointing out that, although botanically very similar to the Cephalotaxaceas, Taxaceas contain triterpene alkaloids (taxines), accompanied by non-alkaloid triterpenes, taxanes, which are also of unique structure in nature. Although they are completely different from taxanes in terms of chemical structures and anticancer mechanism of activity, the harringtonines have analogy with taxanes in more than one respect:

- they have cytostatic properties,
- they consist of a polycyclic skeleton, an inactive biosynthetic precursor of the complete structure, onto which is grafted a side chain containing a similar combination of hydrophilic and hydrophobic substituents,
- the polycyclic part of the taxanes (baccatins in the broad sense) and of the harringtonines (cephalotaxines) is relatively abundant in renewable parts of the plant, whereas the active molecules (harringtonines and taxanes) are ten to one hundred times less abundant therein,
- the plum yew (*Cephalotaxus*) is a rare tree, even rarer than the yew (*Taxus*), and is much less ubiquitous than the latter.

It results from the above facts that, following the manner of the semi-synthesis of taxanes by adding a synthetic chain to a 10-deacetylbaaccatin III of extracted origin, the asymmetric semi-synthesis of harringtonines by esterification of a cephalotaxine of natural origin is of considerable medical and economic value. Furthermore, the current population of *Cephalotaxus* is relatively reduced even in their original habitat. Thus, during its importation into Europe for ornamental purposes last century, *Cephalotaxus harringtonia* was already no longer present in spontaneous form in eastern China and in northern Japan. The use of a precursor present in a renewable part of the tree (the leaf) in order to prepare homo-harringtonine semi-synthetically is thus of considerable environmental interest, all the more so since the total synthesis of optically active cephalotaxine has not been achieved hitherto, despite the extensive synthetic studies carried out in this respect (a certain number of laborious syntheses of racemic cephalotaxine containing 10 to 15 steps have, however, been carried out: see bibliographic review above).

Consider that several hundred tonnes per year of this rare and very slow-growing tree (even slower growing than *Taxus* sp.) need to be extracted to satisfy the current market needs for homo-harringtonine (several kilograms per year), whereas the semi-synthesis would consume only a few tonnes of renewable parts of the tree (leaves). Furthermore, homo-harringtonine (HHT) of natural origin currently available on the active principles market is contaminated with its congeners, which, on account of their structural similarity, are very difficult to separate, even by "preparative" high performance liquid chromatography.

First of all, it should be noted that since the use of cephalotaxine itself as a source for semi-synthesis has not yet been economically justified, no process for selectively extracting this substance has been described hitherto. Moreover, among the active compounds, only harringtonine and isoharringtonine have been the subject of American patent applications for their preparation by extraction [R.G. Powell et al., US 3,793,454 and US 3,870,727]. Harringtonine has been the subject of a Japanese patent [JP 58-032,880] and deoxyharringtonine has been the subject of an American patent [US 3,959,312]. As regards the preparation of homo-harringtonine itself, it has been the subject of only a few semi-synthetic studies [T. Hudlicky, L.D. Kwart and J.W. Reed in "Alkaloid: Chemical and Biological Perspectives" (S.W. Pelletier Ed.), Vol. 5, 639 (1987); M.A. Miah, T. Hudlicky and J. Reed in "The Alkaloids", Vol. 51, 199 (1998)], but no patent application has been made regarding a semi-synthesis process or even an extraction process.

Another aspect which gives the present invention an even greater advantage is that cephalotaxine can serve as a springboard for the synthesis of cephalotaxoids and harringtoids which are useful for antitumour (cancerous and non-cancerous tumours), antiparasitic, antifungal, antiviral and antibacterial chemotherapies.



Harringtonines consist of a complex alkaloid polycyclic alcohol (cephalotaxine), esterified with a side chain, in isolation having no more biological activity than cephalotaxine, but essential for the biological activity of the whole. Saponification of the side chain under harsh conditions leads to the cephalotaxine free base and to harringtonic acids. The attachment of the side chains takes place at the end of the biosynthesis. It has been demonstrated that catabolism leading to this reaction could be triggered *in vivo* under the influence of environmental or physiological stress exerted on the plant [N.E. Delfel, *Phytochemistry*, 403 (1980)].

Cephalotaxine, the polycyclic part consisting of 5 fused rings, has a novel arrangement which is unique in nature, i.e. a benzodioxazepine onto which is fused a spiropyrrolidinopentenediol system. Cephalotaxane contains four asymmetric centres: three "asymmetric carbons" and a heterocyclic tertiary aminic nitrogen. The only reactive function is a secondary alcohol located in position 3, the methyl enol ether located in position 2 being potentially sensitive to proton attack. The whole forms a pseudohelical structure encaging the hydroxyl in the tube formed by the tetrahydrazepine. The base cephalotaxine readily forms highly crystallogenic stable salts (for example hydrochlorides and perhydrochlorides).

This alkaloid is relatively insensitive to basic media. On the other hand, several authors describe a certain level of sensitivity to acids and to quaternization of the nitrogen with methyl iodide, leading to a racemization by simultaneous inversion of the 3 asymmetric centres and of the nitrogen [D.J. Abraham, R.D. Rosensten and E.L. McGandy, *Tetrahedron Letters*, 4085 (1969)]. However, a period of several days in solution at pH 1-4 at 20°C leaves this structure intact (personal observation).

This compound and its congeners which are not O-acylated in position 3 are biologically inactive.

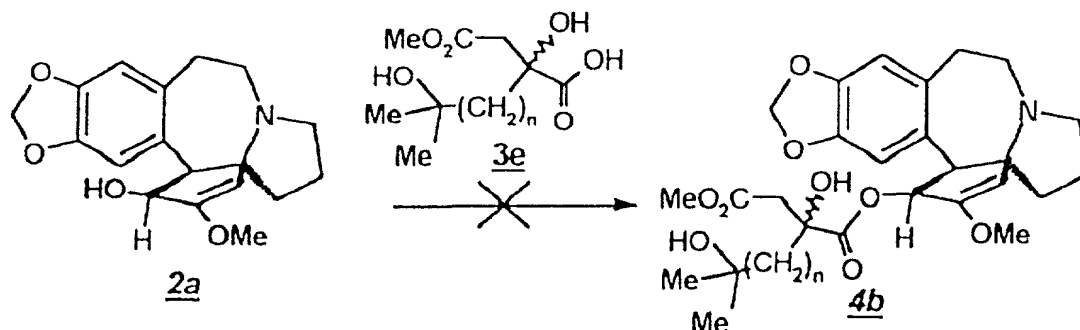
All the side chains for harringtonines which have significant biological activity contain in common the 2-alkyl-2-carbomethoxymethyl-2-hydroxyacetyl unit.

The alkyl chain, of variable length, has at the end either branching constituting an isopropyl bearing (harringtonine HT and homo-harringtonine HHT) or not bearing (deoxy-homo-harringtonine DHT) a tertiary alcohol, or a phenyl radical (for example the neoharringtonine series most recently isolated). In the case of the anhydroharringtonines, the chain can be closed by dehydration between its two tertiary alcohols, for example forming a substituted tetrahydropyran ring. The tertiary carboxyl of this complex diester is borne by the single hydroxyl of the cephalotaxine. The only chiral centre on the side chain is located ( to the ester junction. It contains, besides the first secondary chain, a hydroxyl which, on account of its tertiary nature, does not have the possibility of epimerizing.

Scheme 2 attached shows synthetically the known processes for preparing harringtonines.

Several semi-syntheses of natural cephalotaxine esters and several series of analogues, which have simplified chains but give these analogues reduced cytotoxic activity, have been described hitherto, in particular those of deoxyharringtonine and of isoharringtonine. Most of them relate to simpler and less functionalized esters than those constituting HT and HHT, the esters which are most useful in chemotherapy [for example, deoxyharringtonine, isoharringtonine, T. Hudlicky, L.D. Kwart and J.W. Reed in "Alkaloid: Chemical and Biological Perspectives" (S.W. Pelletier Ed.), Vol. 5, 639 (1987)].

All the literature from 1972 to the present date [Mikolajczack et al., Tetrahedron, 1995 (1972); T. Hudlicky, L.D. Kwart and J.W. Reed in "Alkaloid: Chemical and Biological Perspectives" (S.W. Pelletier Ed.), Vol. 5, 639 (1987); M.A. Miah, T. Hudlicky and J. Reed in "The Alkaloids", Vol. 51, p. 236 (1998)] mention the impossibility hitherto of esterifying the highly sterically hindered secondary hydroxyl of cephalotaxane **2a** with the tertiary carboxyl of the alkanoyl chain of harringtonic acid **3e** totally preformed to give a harringtonine **4b**, i.e. the conversion **2a** + **3e** (**4b**) as described in the example featured in the scheme below

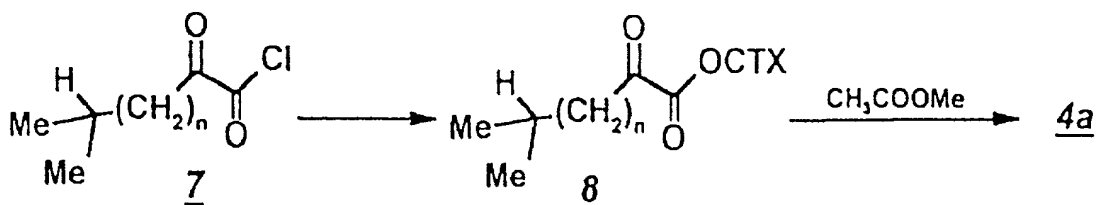


Most of the syntheses described hitherto thus involve binding of the secondary side chain  $-\text{CH}_2\text{CO}_2\text{Me}$ , i.e.:

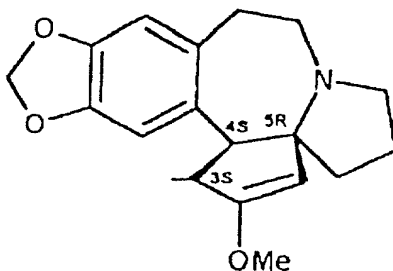
1st) by the Reformatsky reaction between methyl bromoacetate and the carbonyl (real or potential) on the side chain prebound to cephalotaxine, in the presence of zinc, or

2nd) by prior formation of an organolithium reagent.

All the syntheses described thus consist in esterifying cephalotaxine using the ( $\gamma$ -keto alkanoyl chloride 7 lacking the end hydroxyl and containing neither the secondary chain located ( $\gamma$  to the tertiary carboxyl, nor the tertiary hydroxyl ( $\gamma$  to the carboxyl, to give 8 which is then converted into a harringtonine 4a, according to the reaction described below.



In formula 8, CTX- represents the cephalotaxyl radical of formula:



It should be noted that this (-hydroxyalkylation, which at the same time creates the chiral centre on the side chain, has never been achieved asymmetrically.

5

A few synthetic routes involve an esterification of cephalotaxine with a substituted hemisuccinyl chloride, optionally followed by subsequent introduction of the tertiary hydroxyl(s).

- 10 No O-acylation of cephalotaxine, using totally preformed and functionalized chiral chain precursors (to the tertiary carboxyl, has thus been achieved hitherto [T. Hudlicky, L.D. Kwart and J.W. Reed in "Alkaloid: Chemical and Biological Perspectives" (S.W. Pelletier Ed.), Vol. 5, pages 661 to 675 (1987); M.A. Miah, T. Hudlicky and J. Reed in "The Alkaloids", Vol. 51, pages 224 to
- 15 236 (1998)].

Consequently, the methods for preparing harringtonines by semi-synthesis, which have been described to date in the existing art, have the following drawbacks:

- 20 - absence of stereoselectivity,  
 - poor convergence,  
 - mediocre yields,  
 - functionalization and construction of the chain on a rare and expensive substrate,
- 25 - chiral homo-harringtonine not obtained to date.

Since cephalotaxine is present in nature in partially racemized form [personal observation; Huang et al., Scientia Sinica, Vol. XXIII, 835 (1980)], the processes of the prior art which use a natural cephalotaxine as starting

material can only theoretically result in partially racemized harringtonines. The present invention thus has the advantage of obtaining enantiomerically pure harringtonines even from racemic cephalotaxine, since:

5 1st) the asymmetric centre on the side chain is created prior to the esterification step, i.e. the side chain precursor can be obtained in enantiomerically pure form prior to being attached,

2nd) the diastereoisomers obtained in the case of a racemic cephalotaxine  
10 can be separated by chromatography.

The present invention consists in:

- esterifying the hindered free alcohol of a cephalotaxine or alternatively the corresponding metal alkoxide, using a chain in the form of a suitably substituted tertiary carboxylic oxacycloalkane acid which is totally preformed  
15 both in terms of the skeleton and in terms of the functionalization, in order to prepare anhydro-homo-harringtonic acids by semi-synthesis.
- opening the cyclic side chains thus formed in order to obtain the corresponding diols, i.e. the harringtonines (defined above).
- describing a new preparation for all of the diastereoisomers of the  
20 dihydroxylated side chains of the harringtonines in a dehydrated cyclic form (anhydroharringtonic acids) or in which the two hydroxyl groups are protected together by difunctional protecting groups forming a ring.
- resolving all of the harringtonic and anhydroharringtonic acids, in order to couple them separately with the cephalotaxines.

25

One part of the present invention thus consists in synthesizing, in particular, anhydroharringtonine, harringtonine, anhydro-homo-harringtonine and homo-harringtonine.

30 The present invention also relates to esterifying cephalotaxines or metal alkoxides thereof with N-alkyl- and N-carbamoyl-2-alkylisoserine.

Following observations and comparative studies carried out in the taxane series, it was found that, despite a steric bulk which is still greater than that

for the hydroxyl of the cephalotaxines, the hydroxyl located at position 13 on the taxane skeleton made it possible to receive acylation with a relatively bulky chain such as, for example, an N-benzoylphenylisoserine protected in position 2' (although the attempts to acylate baccatin protected with a chain bearing an ( hydroxyl group protected with a benzoyl group all failed).

An experimental study of acylation with a very bulky chain, such as pivalic acid, demonstrated the impossibility of acylating the hydroxyl located at position 13 of a baccatin protected by the dialkyl carbodiimide method, whereas the same reaction performed on cephalotaxine showed easy coupling of the pivaloyl chain.

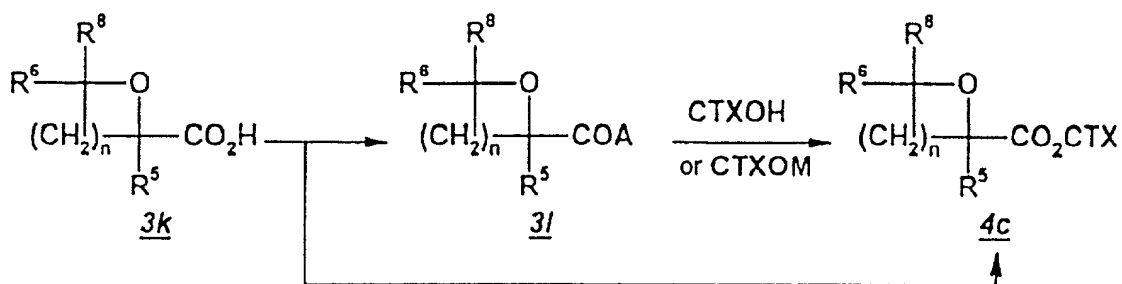
It has also been demonstrated, again in the taxane series, that the improvement in the dynamic compactness of the alkanoyl or aralkanoyl chain by a bifunctional cyclic protection greatly facilitated the coupling. Thus, for example, French patent applications [J.P. Robin et al., FR 95/12739 and FR 95/15557] indicate that the suitably protected, linear N-benzoylphenylisoserines react in several hours at 80°C with formation of epimerization products, the oxazoline or oxazolidine cyclic precursors reacted in less than one hour at 25°C.

The use of the same conditions as above with a chain which has undergone a prior dehydrating cyclization, allowed us to acylate the cephalotaxine or its alkoxides in a few hours at room temperature.

The ease of this acylation was all the more surprising since many authors have designed syntheses which have the drawbacks mentioned above, on the basis of the observation of an impossibility of acylating, explained by the steric hindrance at the two sites: the hydroxyl of the cephalotaxine and the tertiary carboxyl of the side chain precursor [Mikolajczack et al., Tetrahedron, 1995 (1972)].

According to a preferred embodiment or process according to the invention, the hydroxyl function of a cephalotaxane is esterified with a 2-carboxyl-2-alkyl-1-oxacycloalkane derivative.

- 5 The hydroxyl of a cephalotaxane whose skeleton corresponds to the general formula 1 or, more particularly, a secondary alcohol located in position 3 of a cephalotaxane or metal alkoxides thereof, corresponding respectively to the general formulae CTX-O-H and CTX-O-M, M being a metal and CTX- the cephalotaxyl radical defined above, are esterified with the tertiary carboxylic  
10 acid function of a substituted cycloether corresponding to the general formula of the type 3k, or alternatively, preferably, with one of its activated forms, isolated or formed *in situ*, corresponding to the general formula of the type 3l to give 4c, according to the reaction below:



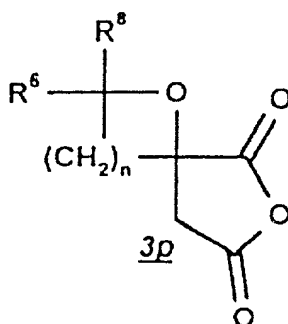
- 15  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are, independently of each other,
- a hydrogen,
  - a linear or branched and/or cyclic, saturated, unsaturated or aromatic, hydrocarbon-based radical, in particular an alkyl, an alkenyl, an alkynyl, a  
20 cycloalkyl, a cycloalkenyl, an aryl, a heterocycloalkyl, the said hydrocarbon-based radical bearing or not bearing (a) heteroatom(s), it being possible for  $\text{R}^5$  and  $\text{R}^8$  to be linked together to form a ring
  - an oxygenated ether bearing one of the above radicals.
- $n$  is between 0 and 8.

25

A is a leaving group capable of leaving behind a carbocation, 3l can thus be, in particular: an acid halide, an anhydride, a mixed anhydride or a cyclic anhydride (when  $\text{R}^5 = -\text{CH}_2\text{CO}-$ ).

The free acid of the type 3k or any activated form of the acids of the type 3l can be used to esterify the cephalotaxanes corresponding to the general formula 1 or, for example, the cephalotaxines of the type CTX-OH.

- 5 Alternatively, the anhydride of 3l can be prepared separately and isolated, and then placed in contact with the alcohol. This is likewise the case for the cyclic anhydrides of the type 3p



- 10 in which  $n$ ,  $R^6$  and  $R^8$  have the same meaning as above, and which can be readily prepared from the corresponding diacids in order once again to give 3k by esterification of methanol or alternatively to become attached as above to the alcohol function of a cephalotaxine of the type 2 with, however, a poorer yield than that above, the primary acid function then
- 15 being methylated conventionally using methanol in the presence of a protonic acid or a Lewis acid, or alternatively using the boron trifluoride etherate/methanol complex or diazomethane.

- 20 Although less effective and more laborious, the method using the acid chloride 3k gave the desired ester 4c.

- All the reagents of the type 3k, 3l and 2, as well as the resulting esters of the type 4c, can be used alone in enantiomerically pure form, or in the form of a racemic mixture or in the form of diastereoisomeric mixtures. The
- 25 intermediates can, in certain cases, not be isolated or formed *in situ* fleetingly.



The reaction can take place at between 0°C and 140°C, with or without an organic solvent, it being possible for these solvents to be alone or as a mixture.

- 5 The esterification of the hydroxyl of cephalotaxane with a 2-carboxyl-2-alkyl-1-oxacycloalkane derivative can be carried out either by acyl transfer to the alcohol or by the carbodiimide method.

10 The esterification reaction by acyl transfer to the alcohol is advantageously carried out according to six specific modes:

- (a) esterification of the free acid with the alcohol in acid catalysis,
- (b) esterification by acyl transfer via anhydrides or halides,
- (c) esterification by acyl transfer using activated esters,
- (d) esterification with scandium triflate,
- 15 (e) esterification with boron trifluoride etherate,
- (f) esterification by the thioester method.

20 The esterification (a) takes place by placing the acid of the type 3k and the alcohol of the type 2 in contact in solution in a co-solvent and in the presence of an acid catalyst. The displacement of the equilibrium can be promoted by adding a dehydrating agent or by azeotropic entrainment or by partition between two immiscible solvents, one of which is miscible with the ester formed and the other with water. These various methods can be combined.

- 25 The acid catalyst can be a protonic acid such as, for example, sulphuric acid, hydrochloric acid, boric acid, preferably para-toluenesulphonic acid, or a Lewis acid which may or may not be supported on a polymer, such as, for example, aluminium chloride, chlorotrimethylsilane or, preferably, boron trifluoride etherate. Advantageously, an ion-exchange resin or bisulphate on  
30 graphite can also be used, for example.

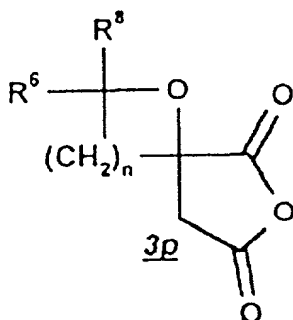
The dehydrating agent can be, for example, a dehydrating agent consisting of an inorganic salt which is low in water and inert with respect to the

reaction, such as magnesium sulphate, sodium sulphate, calcium chloride or, preferably, a molecular sieve.

The esterification (a) uses the same reaction conditions as above. However, in order to result in the formation of the acylium ion characteristic of this method, the ordinary acid catalysts are replaced, for example, with anhydrous sulphuric acid or superacids such as, for example, hydrofluoric acid and its derivatives or antimony pentafluoride.

The esterification (b) consists in using the same physical operating conditions as above, but using 3l, in which A represents a halogen (acid halide), another alkanoyl molecule which is identical (anhydride) or different (mixed anhydride), such as, for example, trifluoroacetyl, 2,4,6-trichlorobenzoyl, formyl, methoxyformyl, sulphonates, phosphates and chlorophosphate.

In a variant of the above method, and in the specific case in which  $R^5 = CH_2CO_2H$ , a cyclic anhydride of the type 3p can be used



in which  $n$ ,  $R^6$  and  $R^8$  have the same meaning as above,

which can be prepared very simply by treating the corresponding diacid with acetic anhydride, for example under the general operating conditions for the preparation of the mixed anhydrides described below.

The catalysis can be acidic, as indicated in the above method, or, preferably, alkaline, for example a tertiary base such as pyridine and/or dimethylaminopyridine (DMAP), pyrrolidinopyridine (PPY), triethylamine, or a stronger base such as a hydride, for example calcium hydride. The solvent

can preferably be an aprotic solvent, for example hexane, toluene, dichloromethane, tetrahydrofuran and/or pyridine.

The esterification (c) is a method similar to the esterification (b). These ester  
5 preparation methods can also be advantageously used and involve, for example, 1-acyloxy-1,2,3-triazole or formamidium or silyl ester or 2-acyloxypyridinium intermediates.

The carbonyldiimidazole method, in which an N-acylimidazole intermediate is  
10 involved, can also be used.

The carbodiimide method uses a dehydrating coupling agent such as a carbodiimide, for example dicyclohexylcarbodiimide (DCC), 1,3-diisopropylcarbo-diimide (DIC) or 1-(3-dimethylaminopropyl)-3-ethyl-  
15 carbodiimide.

The reaction can be catalysed with a tertiary base such as, for example, pyridine and/or dimethylaminopyridine (DMAP), pyrrolidinopyridine (PPY), triethylamine, 4-morpholinopyridine or any other substituted base. N-  
20 Hydroxybenzotriazole (HOBt) or N-hydroxysuccinimide (HO-Su) can also be used, for example.

The molar ratio of 3 relative to 2 can be between 1/1 and 4/1.

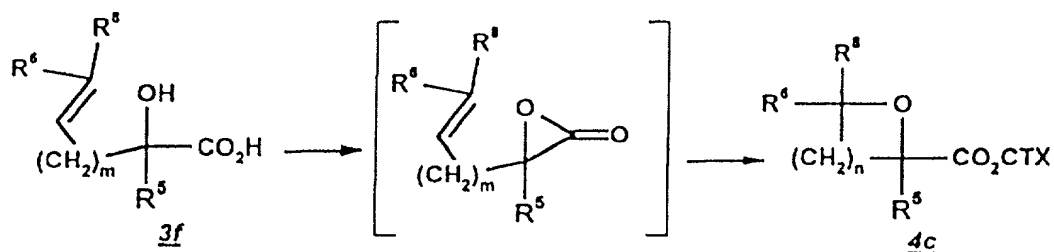
25 The reaction can preferably be carried out under inert gas at a pressure close to atmospheric pressure, preferably at a temperature of between 0°C and about 110°C.

The solvents preferably used are organic solvents such as, for example,  
30 toluene and/or dichloromethane and/or chloroform and/or tetrahydrofuran and/or pyridine and/or dimethylformamide.

The application of the methods described above, for the substituted carboxylic oxacycloalkanes, to the coupling of their synthetic precursors, the

(linear) 1-hydroxy-1-methoxycarbonylmethylalkenecarboxylic acids, made it possible, against all expectation, to synthesize the esters of the type 4c in a single step from ethylenic tertiary (-hydroxy acids of the type 3f instead of the sequence 3f ( 3k ( 3l ( 4c.

5



where m is between 0 and 3,

in formula 4c, n, R<sup>5</sup>, R<sup>6</sup>, R<sup>8</sup> and CTX- have the same meaning as above.

- 10 Indeed, the ethylenic tertiary (-hydroxy acid 3f treated under conditions similar to those above directly gave the cyclic ester of cephalotaxines of the type 4c without isolation of an intermediate. In the case of the method involving a mixed anhydride 3l, the *in situ* formation of the corresponding (-lactone has been assumed on account of the presence of an infrared band at
- 15 1840 cm<sup>-1</sup>.

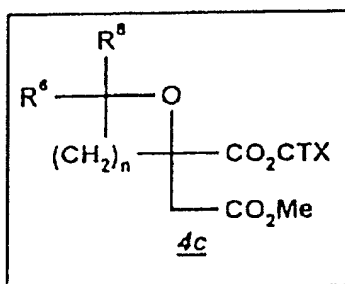
- These elements were confirmed by the formation and isolation of 3l, using 3f only under the activation conditions described above, i.e. to form the mixed anhydride, for example in the presence of 2,4,6-trichlorobenzoyl chloride, or
- 20 alternatively, for example, in the so-called DCC method mentioned above.

- The substituted carboxylic cycloethers of the type 3k, the substituted ethylenic tertiary (-hydroxy acids of the type 3f, their activated intermediates, and cyclic anhydrides of the type 3p, can be coupled with the cephalotaxines,
- 25 either in the racemic series, or, more advantageously, in the optically active series.

In the case of coupling between one of the above types of acid, in the form of racemic mixtures, with a single enantiomer of a cephalotaxine, a relative

stereoselectivity has been found due to the chirality and the major steric hindrance of the reaction site, in the sense that the ratio between each of the two diastereoisomers is generally other than 1.

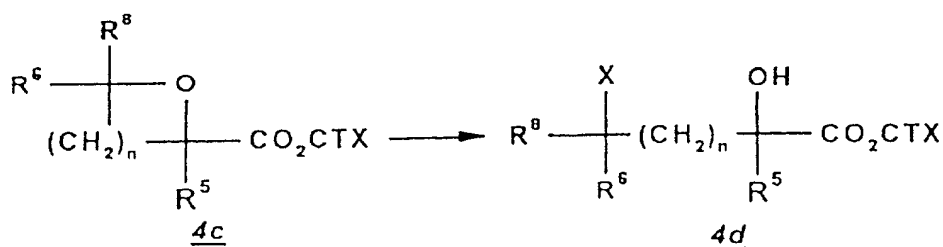
- 5 The separation of the two diastereoisomers formed of the type 4c ( $R^5 = CH_2CO_2Me$ ), known as "anhydroharringtonine"



- in which  $n$ ,  $R^6$ ,  $R^5$  and CTX- have the same meaning as above,  
 can be carried out by preparative chromatography either in a so-called  
 10 normal phase, for example on native silica gel as stationary phase and a  
 mixture of organic solvents as mobile phase, or, preferably, in a reverse  
 phase, for example an inert silica grafted with apolar groups such as, for  
 example, organosilyl, cyanoalkyl, phenylalkyl, preferably  
 octadecylalkylsilane, chains and a mixture of aqueous solvents as mobile  
 15 phase.

- In the case of an enantiospecific coupling, no trace of epimerization is  
 observed on any of the original parts, and the only diastereoisomer obtained  
 can be crystallized. When this diastereoisomer is not crystalline, it is  
 20 chromatographed by flash chromatography in order to remove the reagent  
 residues, and is then precipitated by addition to a non-solvent, in order to be  
 isolated in the form of amorphous powder.

- The opening of the cephalotaxine carboxylate cycloethers of the type 4c to  
 25 give the halo alcohol 4d is shown in the following scheme.

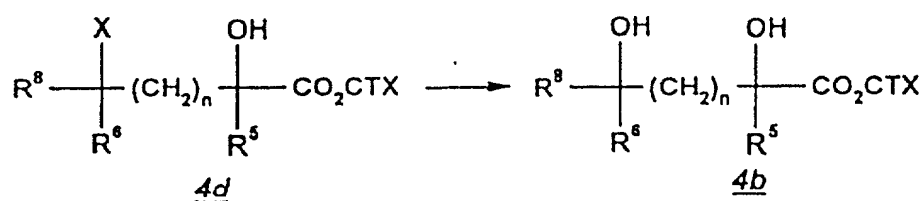


In these formulae n, R<sup>5</sup>, R<sup>6</sup>, R<sup>8</sup> and CTX- have the same meaning as above, X being a heteroatom such as a halogen.

- 5 The cyclic ethers of the type 4c can, in certain examples, have the particular feature of simultaneously containing a methyl enol ether, for example in position 2 of the cephalotaxines. Despite the usual inertia of true cyclic ethers (i.e. non-hemiacetal ethers), the placing in contact of a solution of 4c in an organic solvent, preferably a chlorinated solvent such as, for example,
- 10 dichloromethane, dichloroethane or chloroform, under controlled conditions, i.e. in the presence, for example, of a dilute hydrohalic acid, preferably hydrobromic acid in acetic acid, at low temperature, or else in the presence of a halo-trialkylsilane or alternatively of a boron trihalide, for example boron tribromide at low temperature in an organic solvent, preferably a chlorinated
- 15 solvent, such as, for example, dichloromethane, dichloroethane or chloroform, allowed it to be selectively opened to give a halo alcohol of the type 4d such that X = halogen, with a quantitative yield without any appreciable formation of the O-demethylation product, even in the case of cephalotaxines bearing enol ether(s) or other functions sensitive to ether-
- 20 cleaving agents. In any case, in the event of an accidental demethylation, the enol can easily be selectively remethylated as described in the literature (for example by dissolution in methanol in the presence of para-toluenesulphonic acid).
- 25 The halo alcohols of the type 4d such that X = halogen are of great interest as substrates necessarily leading to heteroatomic analogues, on account of the very good reactivity towards the halogen substitution they bear.

Another variant consists in placing the product of type 4c in contact with an aqueous acid optionally in a miscible or immiscible co-solvent. The acid can be, for example, protonic, and in this case it is an organic or inorganic acid, preferably hydrochloric, tetrafluoroboric or formic acid. In this case, the diols of the type 4b can be isolated directly without passing through the halo alcohol stage described above.

The halo alcohols of the type 4d such that X = halogen are hydrolysed according to the following scheme:



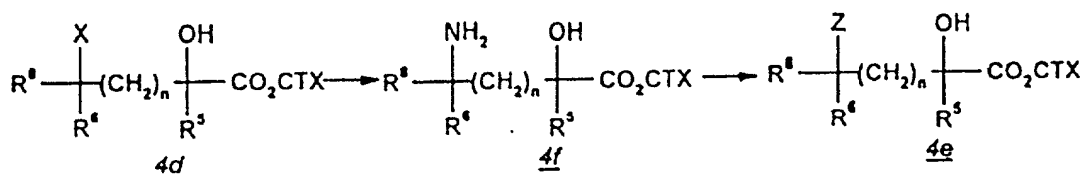
in which n, R<sup>6</sup>, R<sup>8</sup>, R<sup>5</sup>, X and CTX- have the same meaning as above.

The halo alcohols of the type 4d are particularly suitable for controlled hydrolysis by placing in contact with an aqueous inorganic base such as, for example, dilute sodium hydroxide, sodium carbonate or, preferably, barium carbonate, at a temperature of between 0°C and 30°C with stirring, to give the diols of the type 4b.

One variant consists in carrying out the *in situ* hydrolysis of the intermediate halo alcohols of the type 4d at the end of the reaction, preferably carried out by treating the cyclic ether of the type 4c in dichloromethane in the presence of hydrobromic acid in acetic acid, and by directly adding the water or the hydrolysis solution to the reaction medium while cold and with vigorous stirring.

The process according to the invention is particularly suitable for the preparation of azaharringtonines, nitrogenous analogues of the harringtonines.

In order to demonstrate, for example, the flexibility of use of halo alcohols of the type 4d such that X = halogen as substrates, these materials were, for example, subjected to azidolysis by treatment using, for example, an alkaline azide in a solvent such as, for example, ethanol, methanol or dimethylformamide, which, by hydrogenolysis in solution in an organic solvent such as, for example, an alkanol or a lower ester, lead to the corresponding amino alcohol corresponding to the formula 4f (aminodeoxyharringtonine). The amine can then be subjected to amidation under the Schotten-Baumann conditions, i.e. in aqueous media in the presence of an inorganic base as catalyst, to give an amido alcohol of the type 4e



such that Z = NHCOR or NHCOAr, R and Ar being as defined above, more specifically alkyl or aryl groups, respectively, which may or may not be substituted. The amine 4f can also be sec-alkylated to give an alkylaminodeoxyharringtonine (Z = NHR or Z = NHar or Z = NR<sub>2</sub> or Z = NHar<sub>2</sub>, it being possible for the two radicals R and Ar to be identical or independent), or acylated to give amides (Z = NHCOR or Z = NHCOAr) or carbamates (Z = NHCOOR) derived from the corresponding aminodeoxyharringtonine 4f,  
 n, R<sup>5</sup>, R<sup>6</sup>, R<sup>8</sup>, R<sup>9</sup>, X and CTX- being defined as above.

Alternatively, the cyclic ethers of the type 4c can be suitable for the Ritter reaction in the presence of a nitrile (which can serve as solvent) at a low temperature of between -100°C and +30°C in the presence of an acid such as sulphuric acid, perchloric acid or, preferably, tetrafluoroboric acid, to give an acylaminodeoxyharringtonine (Z = NHCOR or Z = NHCOAr) derived from the corresponding aminodeoxyharringtonine 4f.



The extraction of the cephalotaxines of the type 2 is carried out according to the procedure indicated below.

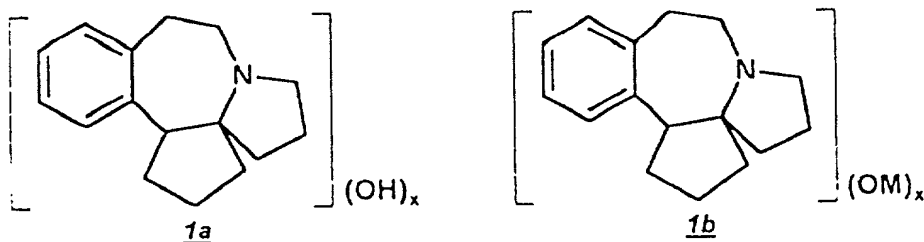
5 The cephalotaxines of the type 2 can be prepared according to the methods described in the literature, either by synthesis or by extraction. In the latter case, since no method uses a direct placing in contact of the plant starting material with an aqueous acid, it has been found to be advantageous to describe this in the present invention. The fresh or dry plant starting material is placed in contact for 24 h with an acidified aqueous-organic mixture using  
10 a dilute inorganic acid or a weak organic acid, so as to bring the pH to between 1 and 4, preferably 3. The inorganic acid can be, for example, sulphuric acid or hydrochloric acid and the organic acid can be citric acid, lactic acid or tartaric acid, for example; the organic solvent can be, for example, a lower alkanol, a ketone, tetrahydrofuran or any other water-miscible solvent used in extraction by those skilled in the art. The water  
15 content is between 20 and 80%, preferably 50%. The solution obtained can be directly chromatographed or basified in order to be counter-extracted, since, in contrast with the methods described in the literature, it contains no chlorophyll and/or plant fat. The counter-extraction using a water-immiscible organic solvent such as a lower ester or, preferably, a lower halogenated hydrocarbon, more particularly dichloromethane, gives a mixture of total alkaloids isolated in the form of a white powder. Several methods for purifying *Cephalotaxus* alkaloids exist, but none, in particular in reverse phase, is specifically geared towards the purification of cephalotaxines and  
20 more particularly of the cephalotaxine of formula 2a.  
25

The present process for purifying cephalotaxine, which forms an integral part of the novel process for the semi-synthesis of harringtonines, thus involves reverse-phase chromatography, which has never been used for this purpose.  
30 This reverse-phase chromatography uses as stationary phase, for example, an inert silica grafted with apolar groups such as, for example, organosilyl, cyanoalkyl, phenylalkyl or, preferably, octadecylalkylsilane chains such as those encountered commercially, and a mixture of aqueous solvents as mobile phase, preferably water itself (without organic solvent); the pH is

adjusted, i.e. to between 2 and 4, with an inorganic acid such as hydrochloric or phosphoric or sulphuric acid. It is also advantageous to add an additive such as, for example, aqueous ammonia or triethylamine. According to this process, which is economically very advantageous since it avoids the use of organic solvent and allows the reuse of the stationary phase for virtually hundreds of operations, the cephalotaxine is obtained in a quantitative recovery yield and with a purity of greater than 95%.

The above method makes it possible to obtain not only laevorotatory cephalotaxines naturally present in the plant material, but also racemic cephalotaxines also present in the natural state.

The metal alkoxides, corresponding to the general formula **1b** ( $n = 1$  to  $12$ ) in which M is a metal, more particularly an alkali metal such as sodium, potassium or lithium, or a transition metal, for example zirconium, titanium or zinc, can be obtained by metallation of one or more of the hydroxyls in the mono- or polyhydroxycephalotaxanes corresponding to the general formula **1a** ( $x = 1$  to  $12$ ) and in which M is more particularly an alkali metal or alkaline earth metal or any other metal which can conventionally give rise to the formation of an alkoxide.



This formation of alkoxide is of great value for acylating more readily in this form the hindered hydroxyls of the cephalotaxanes and most particularly for coupling this cephalotaxane with acylating precursors of the side chains, automatically leading to the harringtonines which are the subject of the present invention.

Several methods can be used to metallate the hydroxyl(s) of a cephalotaxane. For example, a metal hydride, an alkylmetal, an amide or, more generally, any agent capable of exchanging or of giving up a metal atom can be used.

5

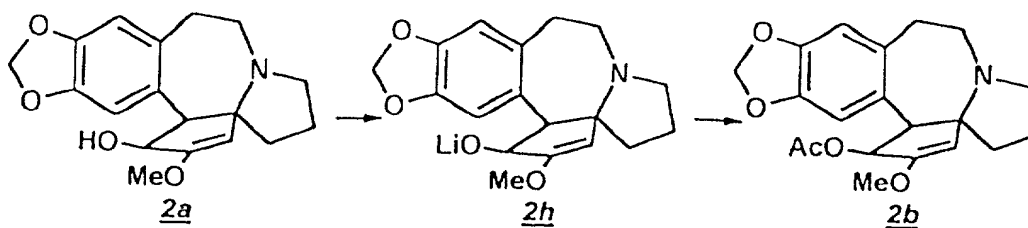
The simple placing in contact of a hydroxylated cephalotaxane in organic solution, preferably under an inert gas, with a metal hydride such as, for example, potassium hydride, lithium hydride or, more particularly, sodium hydride, leads to a cephalotaxane metal alkoxide, which can, for example,  
10 serve as an *in situ* substrate in order to attach, for example, a suitably substituted alkyl, acyl or alkylsilyl group. The organic solvent can be a suitable aprotic solvent such as an ether, more particularly tetrahydrofuran, a liquid aromatic hydrocarbon, preferably toluene or, more generally, any organic solvent which is liquid under the temperature and pressure conditions  
15 used and which has no appreciable reactivity towards the reagent. The temperature of the reaction medium can be between -90°C and +30°C.

The simple placing in contact of a hydroxylated cephalotaxane in organic solution, preferably under inert gas, with a metallated hydrocarbon such as,  
20 for example, a lithiated hydrocarbon, preferably butyllithium, leads to the same metal alkoxides as those above. The same solvents as above can be used, except that, since the reactivity of the metal hydrocarbons is generally greater than that of the metal hydrides, the temperature is between -100°C and -20°C, preferably between -60°C and -80°C.

25

The simple placing in contact of a hydroxylated cephalotaxane in organic solution, preferably under inert gas, with an amide, preferably an alkali metal amide, for example an alkali metal dialkylamide such as lithium dicyclohexylamide or lithium diisopropylamide or alkali metal (lithium,  
30 potassium or sodium) bis(trialkylsilyl)amide leads to the same metal alkoxides as above. The same solvents as above can be

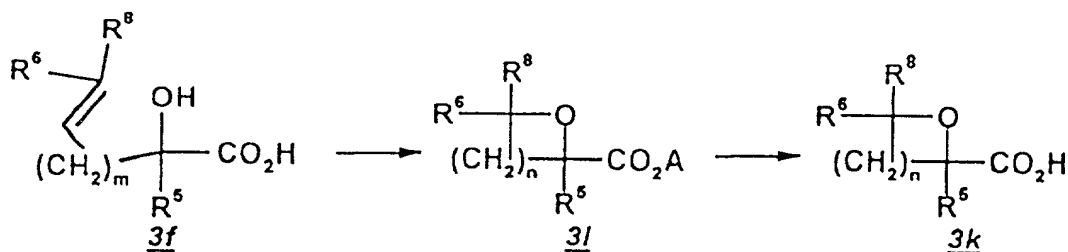
used.



As an example and without detracting from the generality of the present invention, the cephalotaxine **2a** in solution stirred in tetrahydrofuran at  $-70^{\circ}\text{C}$ ,  
 5 treated with one equivalent of butyllithium or lithium bis(trimethylsilyl)amide leads, in a few hours, to the lithium alkoxide **2h**, which, when trapped *in situ* with acetic anhydride, gives the 3-O-acetylcephalotaxine **2b**.

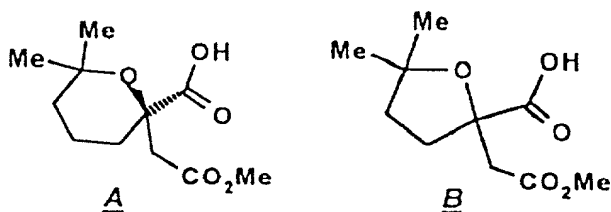
The preparation of the substituted racemic carboxylic cycloethers of the type  
 10 **3k** is detailed below.

According to a first variant, the preparation of these carboxylic cycloethers can be carried out by cyclization of the substituted ethylenic tertiary (-hydroxy acids of the type **3f** according to the following scheme:



15  $m$ ,  $\text{R}$ ,  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^8$  and  $\text{A}$  being defined as above.

The substituted carboxylic cycloethers of the type **3k** such as, for example, **A** or **B** of formulae:



can be prepared from the substituted ethylenic tertiary (-hydroxy acids of the type 3f, by simple dissolution in an organic solvent in the presence of an acid.

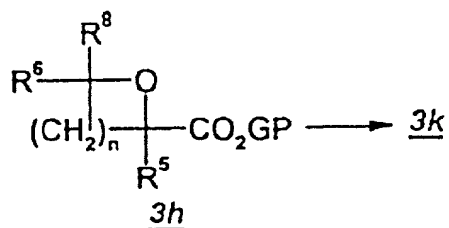
- 5 According to a second variant, the preparation of these carboxylic cycloethers can be carried out by cyclization concomitant with the formation of the acylating species. As mentioned above, acids whose tertiary alcohol is free, such as 3f, cyclize spontaneously by the action of a dehydrating agent required for a certain technique for acylating a cephalotaxine alcohol  
10 mentioned above and then esterifying the latter to give 4c.

In the absence of an alcoholic substrate to receive it, the ethylenic tertiary alcohol of the 3f type leads, under anhydrous operating conditions, to the isolation of the acylating intermediate 3l, mentioned above, or, by hydrolysis,  
15 to the isolation of the acids of the type 3k.

In this case, the procedure used is strictly the one described for coupling involving the formation of an acylating species *in situ* but in the absence of a substrate of the cephalotaxine type.

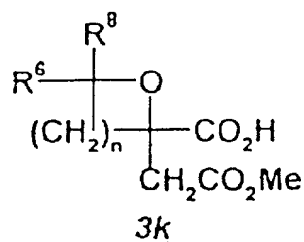
20

According to a third variant, these substituted carboxylic cycloethers of the type 3k can be prepared by deprotecting the tertiary carboxyl of the suitably substituted precursor 3h,

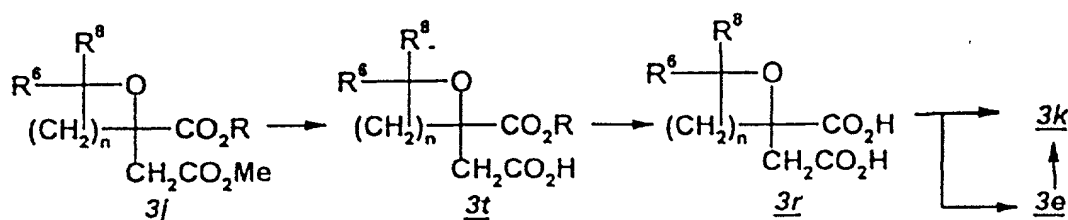


- 25  $\text{R}^5$ ,  $\text{R}^8$ ,  $\text{R}^6$  and  $n$  being defined as above and GP representing a protecting group for the acids, with, as a specific case,  $\text{GP} = \text{R}$ .

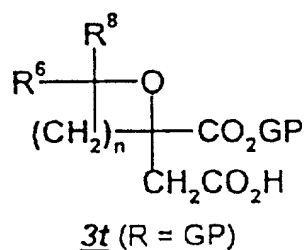
According to a fourth variant, in the specific case in which  $R^5 = \text{CH}_2\text{CO}_2\text{R}$ , the suitably substituted carboxylic cycloethers of the type 3k such that  $R^5 = \text{CH}_2\text{CO}_2\text{R}$  below,



- 5  $\text{R}^8$ ,  $\text{R}^6$  and  $n$  being defined as above, can be prepared by total saponification of the corresponding diesters 3j such that  $R^5 = \text{CH}_2\text{CO}_2\text{R}$ , followed by mild selective methylation of the intermediate diacid 3r such that  $R^5 = \text{CH}_2\text{CO}_2\text{H}$ .



Incidentally, this process, carried out at room temperature and with rigorous monitoring of the reaction kinetics, leads to selective saponification of the above primary ester; it thus gives access to derivatives of the type 3t such that  $\text{R} = \text{GP}$  below,



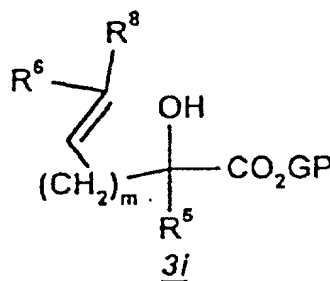
$\text{R}^8$ ,  $\text{R}^6$ ,  $\text{GP}$  and  $n$  being defined as above, which can be coupled with the cephalotaxines using the methods described above in order to ensure the absence of transesterification during the coupling of the tertiary acids which form the subject of the present invention.

According to a fifth variant, the preparation of these carboxylic cycloethers can be carried out by regioselective methanolysis of the corresponding cyclic anhydride.

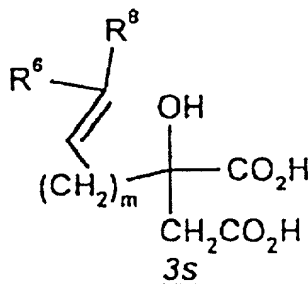
- 5 As mentioned above, the diacid 3r leads, by self-dehydration, to the cyclic anhydride 3p, which is a good acylating agent for alcohols, such that by methanolysis 3k is also preferentially obtained such that  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$ , which constitutes an additional preparation method.
- 10 The preparation of the substituted ethylenic tertiary (-hydroxy acids of the type 3f is detailed below.

The substituted ethylenic tertiary (-hydroxy diacids which are the precursors of the monoacids of the type 3f can, like their cyclic analogues of the type 3k above, be obtained:

- either by selective deprotection of their precursors of the type 3i

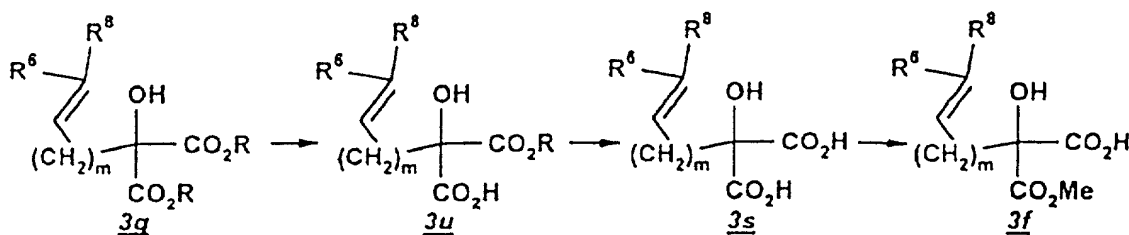


- 20 - or, for example, in the case of the diacids of the type 3s such that  $R^5 = \text{CH}_2\text{CO}_2\text{H}$ ,



by selective methylation of the primary carboxyl in particular, by placing the reactants in prolonged contact in methanolic solution at room temperature or using the boron trifluoride/methanol complex.

- 5 The diacids of the type 3s such that  $R^5 = CH_2CO_2H$  can be obtained by saponification of the corresponding diesters of the type 3g such that  $R^5 = CH_2CO_2Me$  by placing the latter in contact with an excess of base in an aqueous or aqueous-alcoholic medium,



m, R, R<sup>8</sup> and R<sup>6</sup> being defined as above.

- 15 The alcohol can be a lower alcohol such as methanol, ethanol or, preferably, isopropanol, and the base can be, for example, an alkali metal or alkaline earth metal base or a rare earth metal hydroxide or aqueous ammonia. When the reaction takes place at a temperature of between 0°C and 30°C for 15 minutes to 1 hour, the regioselective saponification of the primary ester can be obtained without any resulting difficulty. By increasing the
- 20 temperature to the boiling point of the solvent mixture and/or by lengthening the reaction time, the diacid is obtained in good yield and without formation of by-products.

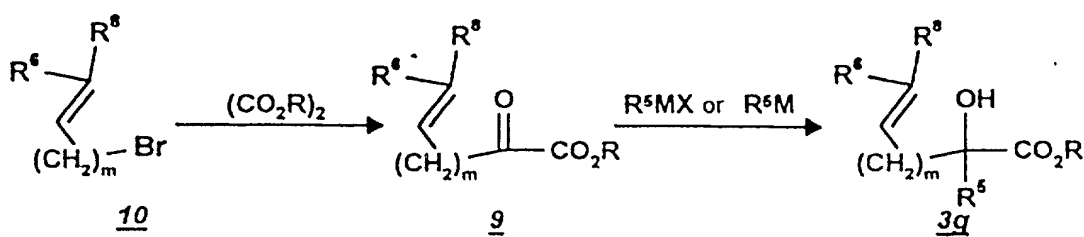
- 25 The diacids of the type 3s such that  $R^5 = CH_2CO_2H$  can, on account of their crystallogenic properties, then generally be obtained in enantiomerically pure form by successive crystallizations of enantiomerically enriched mixtures until a constant optical rotation is obtained.



The diacids obtained above can then be converted into substituted ethylenic tertiary (-hydroxy acids of the type 3f such that  $R^5 = CH_2CO_2Me$  by selective mono-esterification of their primary carboxyl, using methanol in the presence of a protonic acid or a Lewis acid or alternatively using the boron trifluoride etherate/methanol complex or diazomethane.

Incidentally, and as for the cyclic analogues of the type 3f such that  $R^5 = CH_2CO_2H$ , above, this process carried out at room temperature and with rigorous monitoring of the reaction kinetics leads to selective saponification of the above primary ester; it thus gives access to derivatives of the type 3u such that  $R^5 = CH_2CO_2H$ , which can be coupled to the cephalotaxines using the methods described above in order to ensure the absence of transesterification during the coupling of the tertiary acids which form the subject of the present invention.

The substituted ethylenic tertiary (-hydroxy esters of the type 3g can be prepared according to the scheme outlined below:



$R^6$ ,  $R^8$ ,  $m$  and  $R^5$  having the same meaning as above.

The ethylenic esters of the type 3g can be prepared according to the numerous methods described in the literature for similar cases such as, for example, (-hydroxyalkylation of the corresponding 1-alkyl- or 1-alkenyl-1-keto ester of the type 9.

As an example and without removing anything from the generality of the present invention, the (-hydroxyalkylation of the 1-alkenyl-1-keto ester of the type 9 with the lithium methoxycarbonylmethyl enolate ( $R^5M =$

MeOCOCH<sub>2</sub>Li) or of the corresponding organozinc reagent (Réformatsky reaction, in which R<sup>5</sup>MX = MeOCOCH<sub>2</sub>ZnBr) leads to the diester 3g such that R<sup>5</sup> = CH<sub>2</sub>CO<sub>2</sub>Me.

- 5 The same reactions applied to a chiral ester (R = R\*) lead to a mixture of separable diastereoisomers which, after deprotection of the tertiary acid function, each lead to the diastereoisomer of the pair.

10 Moreover, the (-hydroxyalkylation reaction of the 1-alkyl- or 1-alkenyl-1-keto ester of the type 9, conducted in the presence of a chiral inducing agent such as sparteine or quinine, can give a significant enantiomeric enrichment, which can be further enhanced by fractional crystallization.

15 The keto esters of the type 9 are themselves conventionally obtained by C-semi-acylation of the carbanion of the corresponding alkyl or alkenyl halides of the type 10 with a dialkyl oxalate.

20 One of the advantages over the prior art of the synthetic process which forms the subject of the present invention lies in the possibility of coupling an entirely preformed chain with the cephalotaxines. Thus, the preparation of the above anhydroharringtonic acids in enantiomerically pure form 3k is of considerable interest, since the post-coupling creation of the chiral centre in position 2' of the harringtonines during the attachment of the secondary chain as described in the prior art leads to an epimeric mixture, on the one hand, 25 which is very difficult to separate, and, on the other hand, to a loss of about 50% of the very precious cephalotaxines (not recyclable in a process for manufacturing a medicinal substance using Good Manufacturing Practice).

30 Several methods have been used to achieve this aim. They all apply both to the cyclic monoacids of the type 3k or to their diacid precursors of the type 3r, and to their ethylenic linear precursors of the type 3f, it being possible for chiral chromatography methods also to be applied to the precursors which have no function capable of engaging a reversible chemical bond with a chiral species (in this instance free acid functions).

According to a first step of the process for the enantiospecific preparation of these acids, an epimeric mixture is formed by combination with a chiral alcohol or amine.

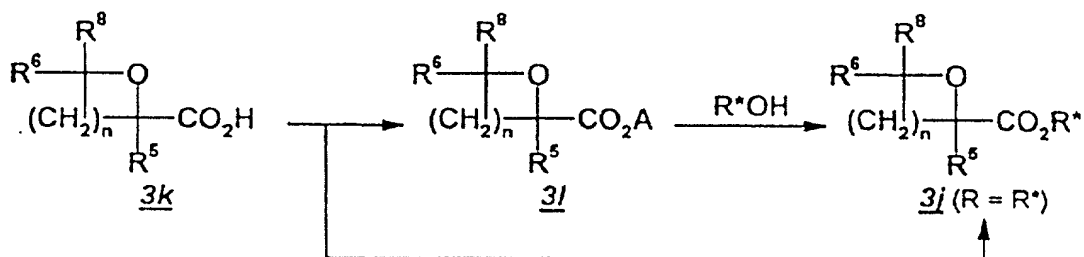
5

The reactions for esterifying a hindered secondary alcohol function of a cephalotaxine with oxacycloalkanecarboxylic acids of the type 3k above (including those formed *in situ* from ethylenic tertiary (-hydroxy acids of the type 3f) can also be applied to the esterification of another chiral alcohol in order to convert a racemic mixture, or one with partial enantiomeric enrichment of acids of the type 3k, into a diastereoisomeric mixture on which all of the non-chiral separation methods become applicable. The above methods are also applicable without modification to the amidation of chiral primary or secondary amines.

15

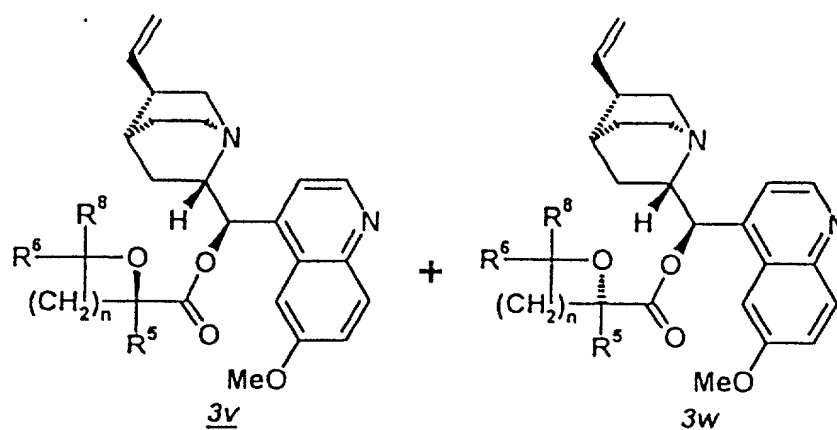
Thus, when the oxacycloalkanecarboxylic acids of the type 3k or their ethylenic linear precursors are reacted with a chiral alcohol, denoted by  $R^*OH$ , or an amide  $R^*R^*NH$  (it being possible for  $R^*$  to be replaced with a hydrogen), two chemical species are obtained in which the physicochemical properties are distinct (for example NMR, melting point, solubility, chromatographic properties, enzymatic or microbiological attack, etc.). The alcohol or the amide must preferably be hindered and bear their chiral centre at their site of binding with the tertiary carboxyl of the acid of the type 3k. The alcohol can be, for example, menthol, borneol, valinol or, preferably, quinine. The amine can be, for example, ephedrine; more generally, any commercial chiral alcohol or amide can be used.

25



$n$ ,  $R^5$ ,  $R^6$ ,  $R^8$  and  $A$  having the same meaning as above,  $R^*$  having the same meaning as  $R$ , but being chiral.

As an example, and without detracting at all from the generality of the present invention, (-)-quinine, which, like the cephalotaxines, is an alkaloid with a sterically hindered secondary alcohol function, reacts with the racemic mixture of the oxacycloalkanecarboxylic acids of the type 3k to give the mixture of the two corresponding epimers 3v such that  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$  and 3w such that  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$ :



The binary mixtures of epimers obtained by the combination with a chiral compound can be separated, for example, by fractional crystallization, by distillation, by counter-current liquid-liquid partition and, given the high added value of these intermediates, by any common preparative chromatography technique, for example normal phase chromatography, exclusion chromatography, preferably in reverse or normal grafted phase. Since these methods are synergistic, they can advantageously be combined in order to improve the diastereoisomeric purity.

As an example and without detracting at all from the generality of the present invention, the mixture of the two epimers 3v (such that  $n = 3$ ;  $R^6 = R^8 = \text{Me}$ ;  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$ ) and 3w (such that  $n = 3$ ;  $R^6 = R^8 = \text{Me}$ ;  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$ ), cited in the above example, can be separated without difficulty and in quantitative yield using a grafted phase of octadecylsilane type and a methanol/water mobile phase.

The regeneration of the oxacycloalkane- carboxylic acids of the type 3k in enantiomerically pure form can be carried out by total hydrolysis followed by selective remethylation of the primary carboxyl of the suitably selected diastereoisomer (see above sequence 3j ( 3k) or, when it is an ester bond with an oxygen in the benzyl position (see for example quinine above), by simple hydrogenolysis. In the latter type of case, the drawback of the hydrogenolysis is largely offset by the economy of a step on an expensive product.

- 10 As an example and without detracting at all from the generality of the present invention, (-)-quinine (2'R)-anhydroharringtonate 3v (such that  $n = 3$ ;  $R^6 = R^8 = \text{Me}$ ;  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$ ) gave a (2R)-anhydroharringtonic acid of the type 3k and dihydrodeoxyquinine which can thus not be recycled, but this is a minor drawback in view of the low cost of this alkaloid. Alternatively, the double
- 15 saponification of 3v followed by selective remethylation gave a product which was entirely identical to the (2R)-anhydroharringtonic acid of the type 3k above.

- The enantiomer of non-natural configuration (2S) can, after having undergone the same conversions as its (2R) enantiomer, be exploited, for example, for the purposes of structure-activity relationship studies.

- According to a first step of the process for the enantiospecific preparation of these acids, the racemic mixtures are resolved by formation of salts with a chiral basic species.
- 25

- The racemic mixtures of oxacycloalkane carboxylic acids of the type 3k (including those formed *in situ* from ethylenic tertiary (-hydroxy acids of the type 3f), can form a salt with a chiral amine by simple placing together in solution in an organic solvent. Although most of the methods described above for separating the esters and amides formed with 3k are applicable (for example chromatography), since the salts formed are generally highly crystallogenic, it is fractional crystallization which is preferably carried out to resolve the acids of the type 3k. The solvents used, alone or as a mixture,
- 30

can preferably be polar organic solvents which may or may not be combined with water, such as, for example, ketones, alcohols and lower esters. The reaction to form the salt preferably takes place at a temperature of between 0 and 100°C. The recrystallization can be carried out by redissolving the salt in a mixture whose solvent power can be adjusted with precision by means of the use of the above combinations of solvents and by varying the temperature according to the standard techniques practised by those skilled in the art. When the diastereoisomeric enrichment is deemed to be sufficient, the salt is decomposed in the presence, for example, of a dilute aqueous acid such as hydrochloric acid or sulphuric acid. The extraction of the enantiomer of the regenerated acid can be carried out using a water-immiscible organic solvent such as, for example, a lower ester.

As an example and without detracting at all from the generality of the present invention, the racemic mixture of (2R or 2S)-anhydroharringtonic acids of the type 3k can be resolved, for example, by placing them in contact with (-)-ephedrine, followed by fractional recrystallization in an ethyl acetate/methanol mixture.

The 2R-anhydroharringtonic acid of the type 3k is then regenerated by placing the purified salt in contact with 2N hydrochloric acid and continuously extracting the acidic aqueous phase with ethyl acetate.

The oxacycloalkanecarboxylic acids of the type 3k described above can be subjected to preparative chiral chromatography.

The final products are purified by HPLC to give final products for pharmaceutical use.

Despite the performance levels of the modern methods of synthesis, of semi-synthesis and of isolation of natural substances, it is now established in the regulations issued by the health authorities in industrialized countries that impurity levels of greater than one per thousand (0.1% m/m) in a medicinal substance can be detrimental to the patient.

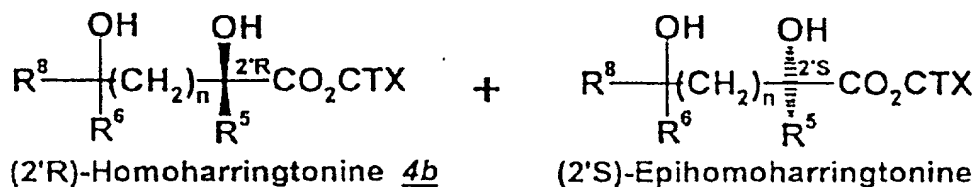
An identification followed by toxicology studies on any toxicologically unknown substance exceeding this threshold is, moreover, systematically demanded, in order to obtain pharmaceutical files for authorization to market the medicinal products.

The diastereoisomeric purity (with, as a specific case, the enantiomeric purity) can, moreover, lead to therapeutic aberrations; for example, it is well known that quinine (see above formula) is an antimalaria agent, whereas one of its diastereoisomers is a cardiac antifibrillant.

In the therapeutic field of the substances forming the subject of the present invention, it is common to encounter multiplications of from 10 to 100 of the active principle or of a side effect by minor changes (involuntary here) in the molecular structures.

Among the methods for achieving this level of purity, industrial high-resolution chromatography occupies a position of choice, its high cost being an argument which carries little weight compared with the very high added value of the sophisticated active principles, the robustness which it gives to the processes and the safety it offers to users.

As an example, and without detracting at all from the generality of the present invention, homo-harringtonine 4b, such that  $n = 3$ ;  $R^6 = R^8 = \text{Me}$ ;  $R^5 = \text{CH}_2\text{CO}_2\text{Me}$ , CTX- = cephalotaxyl,



$R^6$ ,  $R^8$ ,  $R^5$ ,  $n$  and CTX- being defined as above,

can be freed of its epimer at the same time as its other related impurities by preparative reverse-phase chromatography using a grafted reverse phase of octadecylsilane type as stationary phase and a suitably adjusted methanol/water mixture as mobile phase.

5

This process gives a product whose sum of related impurities is less than 0.5% and for which none of these impurities taken individually exceeds 0.1%.

0527006 "03469  
6697E" 9002250



The present invention concerns a process for the preparation of sidechain-bearing cephalotaxane of the following formula and/or a salt thereof

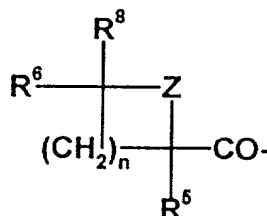


where

5             $\Omega$  ("omega") is a representative radical of the chain terminal moiety and  $\text{-CO-}$  is the carbonyl of the ester group bonded to cephalotaxane;

the  $\Omega\text{-CO-}$  radical is corresponding:

- either to the following substituted heterocycloalkane formula:



10            where n is included between 0 and 8;

Z is oxygen, nitrogen or sulfur heteroatom;

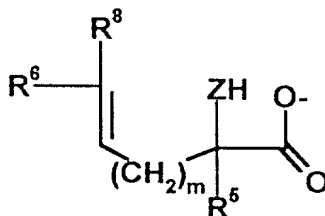
$R^5$ ,  $R^6$  and  $R^8$  are independently

hydrogen;

15            hydrocarbon radical, saturated, insaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl, of said radical including or not heteroatom(s);  $R^6$  and  $R^8$  may be included in a cycle;

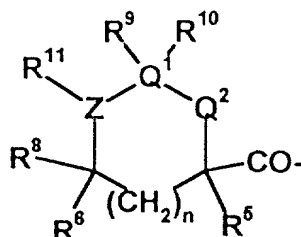
oxygen ether bearing one of the former radicals;

20            - or to the following linear alkene formula:



where m is included between 1 and 8,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above;

- or to the following formula:



where n,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above;

Z and  $Q^2$  are independently oxygen, nitrogen or sulfur heteroatom;

$Q^1$  is carbon, silicon or phosphorus atom;

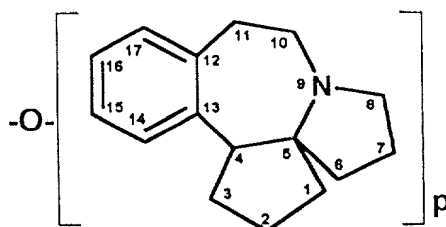
$R^9$  and  $R^{10}$  are independently hydrogen, alkoxy, hydrocarbon radical, including or not heteroatom(s), saturated, unsaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl;

$R^9$  and/or  $R^{10}$  having the ability to be null or taken together to make an heteroatom and/or make a multiple bond with  $Q^1$ ,  $R^9$  and  $R^{11}$  having the ability to be null to make a multiple bond between the two atoms of carbon bearing them; and

$R^{11}$  is hydrogen, arylcarbonyl, alkoxycarbonyl, aryloxy carbonyl or alkylcarbonyl;

where

-O-CTX is cephalotaxine moiety of the following formula a salt thereof:

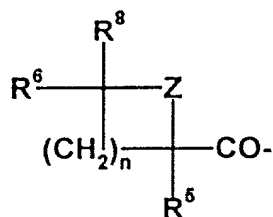


where p is equal to 1 or 2;

the two types of radicals  $-\Omega$  and  $-\text{CTX}$  above-mentioned being bonded with an ester bond  $-\text{CO}-\text{O}-$

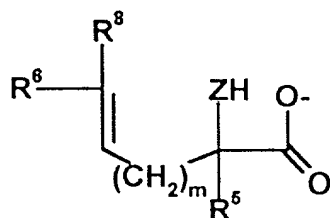
the said process bringing together:

- either carboxylic acid with general formula  $\Omega\text{-CO-OH}$  or a salt thereof;
- or an activated form of an acid with general formula  $\Omega\text{-CO-A}$  or a salt thereof, with  $\Omega\text{-CO}$  of the following formula:



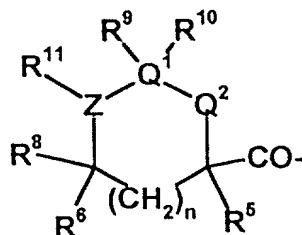
where  $n$ ,  $Z$ ,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined above;

where  $\Omega\text{-CO}$  of the following formula:



$m$  is included between 1 and 8,  $Z$ ,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined above;

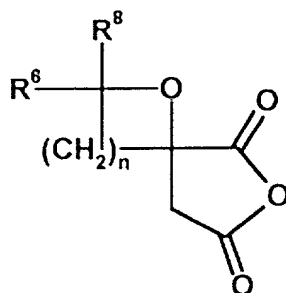
where  $\Omega\text{-CO}$  of the following formula:



and where  $n$ ,  $Z$ ,  $\text{Q}^1$ ,  $\text{Q}^2$ ,  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^8$ ,  $\text{R}^9$ ,  $\text{R}^{10}$  and  $\text{R}^{11}$  are as defined above

A represents:

- either cyclic anhydride of the following formula:

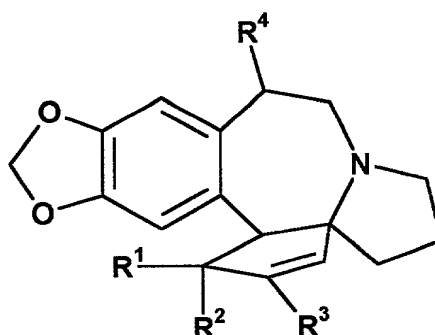


5 where  $n$ ,  $R^6$  and  $R^8$  are as defined above;

the reaction has been completed by methylation of the primary carboxyl thus formed, with:

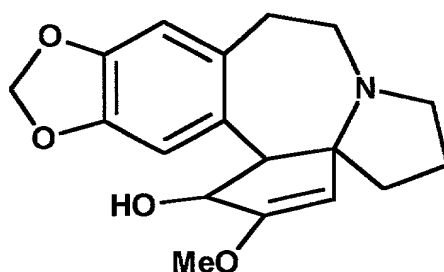
- either a cephalotaxane or a salt thereof, bearing at least a free hydroxyl group, of the formula H-O-CTX, where CTX are as defined above;
  - 10 - or a metallic alcoxide of the formula M-O-CTX, where CTX are as defined above and M is a metal;
  - or an activated form of its hydroxyl group of the formula Y-O-CTX, where -O-CTX is as defined above and Y is, either a leaving group to allow a negative charge on oxygen atom by cleavage between Y- and -O-CTX, or to allow a
  - 15 carbocation by cleavage between Y-O- and -CTX;
- with the possible presence of one or several reaction additives to form said sidechain-bearing cephalotaxane and/or a salt thereof.

20 Most preferably, Z is an oxygen atom and the cephalotaxane H-O-CTX is a cephalotaxine of the following formula, or a salt thereof:



where  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are independently hydrogen, hydroxyl group or alkoxide.

A cephalotaxane H-O-CTX, as defined above, is cephalotaxine, or a salt thereof, where  $R^1$  is hydroxyl,  $R^2$  is methoxyl,  $R^3$  and  $R^4$  are hydrogen.



$R^5$  is preferably hydrogen or  $-\text{CH}_2\text{-CO-O-Me}$ .

The  $\Omega$ -CO radical is preferably such as  $n = 1$  to 4,  $R^6$  and  $R^8$  are methyl.

The  $\Omega$ -CO radical may be too such as  $n = 1$  or 2,  $R^6$  is phenyl and  $R^8$  is hydrogen.

When  $R^5$  is  $-\text{CH}_2\text{-CO-O-Me}$ ,  $R^1 = \text{OH}$ ,  $R^2 = \text{OMe}$ ,  $R^3 = R^4 = \text{H}$ , the cephalotaxane is preferably such as  $n = 0$ ,  $Z$  is a nitrogen atom and  $R^8$  is hydrogen.

A may be  $\Omega$ -CO-CO where  $\Omega$  is defined as above, or an halide.

A may also be a radical of compound  $\Omega$ -CO-A having the ability to generate cleavage of the bond between carbonyl group and substituent A to provide  $\Omega\text{-CO}^+$  and  $\text{A}^-$ .

In addition, A is a radical selected from substituents:

méthoxyformyloxy of formula  $\text{MeOCCO}-$ ,

trifluoroacétyloxy of formula  $\text{CF}_3\text{CCO}-$ ,

alkylsulfonyloxy of formula  $\text{RSO}_3-$ ,

5 phosphoxy of formula  $(\text{RO})_2\text{PO}-$ ,

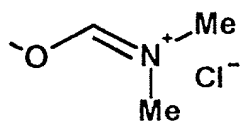
halophosphoxy of formula  $\text{ROP}(\text{Cl})\text{O}-$ ,

trialkylsilyloxy of formula  $\text{R}_3\text{SiO}-$ ,

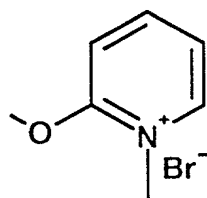
formulas wherein R is alkyl,

diméthyl-formamidinium chloride of formula

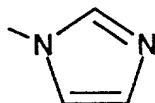
10



or acyloxy-pyridinium bromide of formula



A may also be 2, 4, 6-trichlorobenzoyloxy or a radical corresponding to the following formula:



15

In the case where A is a carbonyl-diimidazole, where A is 2, 4, 6-trichlorobenzoyloxy, the reagent of formula  $\Omega\text{-CO-A}$  is obtained by contacting an acid  $\Omega\text{-CO-OH}$ , as defined above, with 2, 4, 6-trichlorobenzoyloxy carbonyl-diimidazole in presence of a strong base such as an alkoxide.

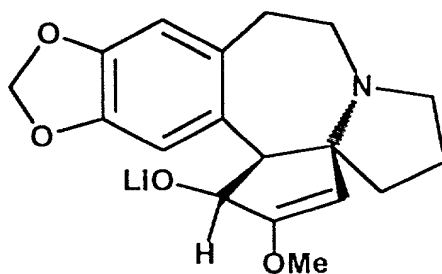
20

According carbodiimide method, the coupling additive is a substituted carbodiimide and/or a basic additive such as tertiary amine for example.

For example, the substituted carbodiimide is selected from cyclohexylcarbodiimide (DCC), 1,3-diisopropylcarbodiimide (DIC) and chlorhydrate of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide.

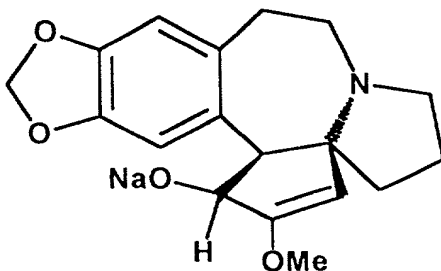
- 5 The cephalotaxine alcoxide, corresponding to the formula **M-O-CTX** where M and CTX are as defined above, may be obtained by contacting a cephalotaxine of formula **H-O-CTX** with metal himself, an amidure, a metallic hydride or an alkyl-metal.
- 10 M may be an alkaline metal such as lithium, potassium or sodium.  
The aim of the present invention is also the preparation of new compounds such as :

- the lithium alcoxide of cephalotaxine corresponding to the following formula:

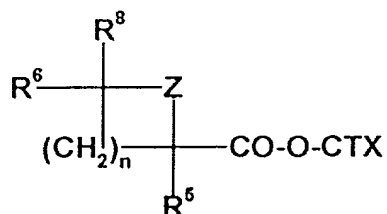


15

- the sodium alcoxide of cephalotaxine corresponding to the following formula:



- a sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:



where

n is included between 0 and 8;

Z is oxygen, nitrogen or sulfur heteroatom;

R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are independently

hydrogen;

hydrocarbon radical, saturated, insaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl, of said radical including or not heteroatom(s);

oxygen ether bearing one of the former radicals;

CTX is as defined above;

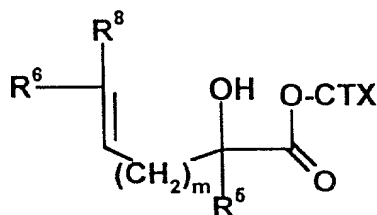
except for compounds where Z is oxygen atom and,

1°) n = 2 or 3, and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OMe or hydroxyl,

1°) n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OMe or hydroxyl;

3°) n = 3 and simultaneously R<sup>6</sup> is hydroxyl, when R<sup>8</sup> is méthyl and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical.

- a sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:





where

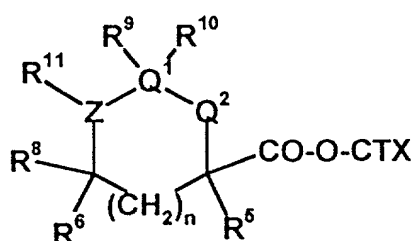
$m$ ,  $R^5$ ,  $R^6$ ,  $R^8$  and CTX are as defined above;

except compound where  $m = 2$ ,  $R^5 = CH_2CO_2CH_3$ ,  $R^6 = R^8 = \text{methyl}$  and CTX is as defined above.

5

$R^5$  is preferably the  $-CH_2-CO-O-CH_3$  radical.

- a sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:



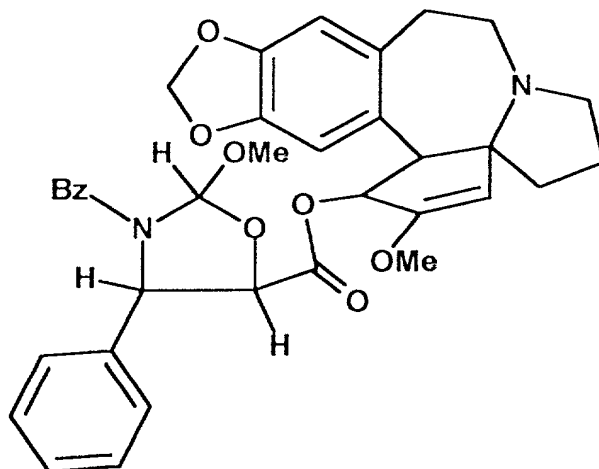
10

where  $n$ ,  $Z$ ,  $Q^1$ ,  $Q^2$ ,  $R^5$ ,  $R^6$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$ ,  $R^{11}$  and CTX are as defined above.

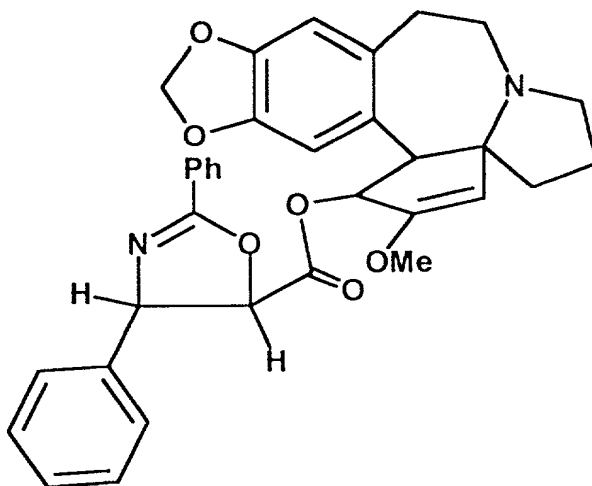
Preferably,  $Q^2$  is oxygen atom and/or  $Z$  is nitrogen atom and the cephalotaxane such as  $n = 0$ .

15

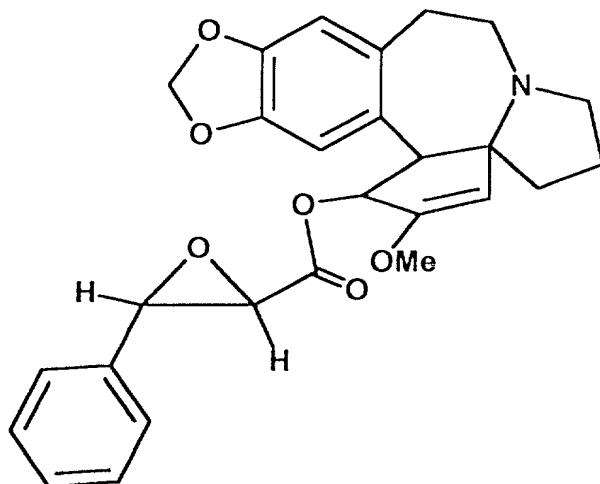
- a sidechain-bearing cephalotaxane corresponding to the following formula:



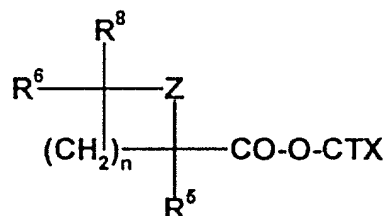
- a sidechain-bearing cephalotaxane corresponding to the following formula:



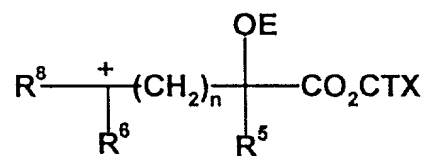
5 - a sidechain-bearing cephalotaxane corresponding to the following formula:



When the cyclic side-chain of sidechain-bearing cephalotaxane, and/or a salt thereof, presents the following formula:



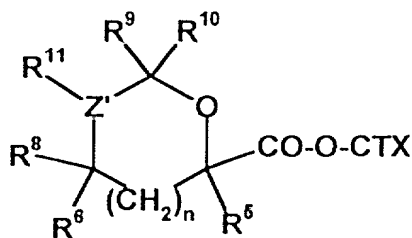
where  $n$ ,  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^8$ , CTX and Z are as defined above, the said chain is open with an agent and/or a protonic or not protonic electrophilic radical E in aqueous or not aqueous medium, to provide an intermediate compound of the following formula:



where  $n$ , CTX,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined above, E is either hydrogen or the provisionally or definitively fixed electrophilic radical;

the aforementioned intermediate compound may be attacked with an agent or a nucleophilic radical Z', deliberately added or possibly present in the medium, and

when the cyclic side-chain of sidechain-bearing cephalotaxane, and/or a salt thereof, presents the following formula:



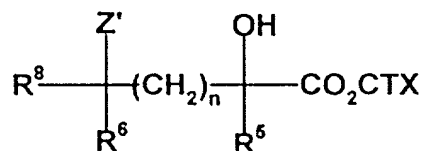
where  $n$ ,  $R^5$ ,  $R^6$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  are as defined above, and  $Z'$  is an heteroatom;

5

the said chain is open by hydrolysis or carefully solvolysis with possibly presence of activation and/or opening additive.

In addition, to provide an open sidechain-bearing cephalotaxane of the following formula:

10

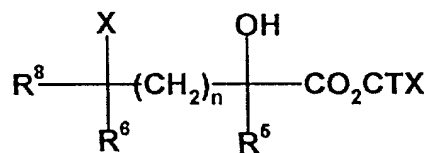
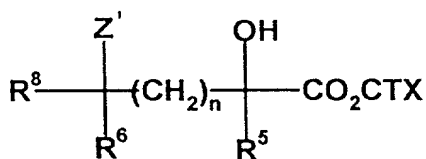


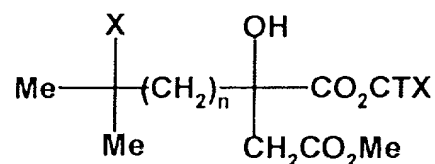
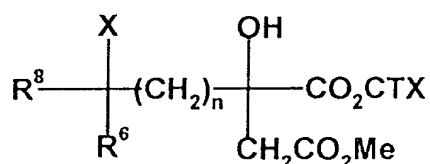
where  $n$ ,  $CTX$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above;

$Z'$  is:

- either a halogen or an heteroatom bearing a hydrogen or a radical  $R^{11}$  such as defined above;
- 15 - or an hydrogen, hydrocarbon radical, the said radical bearing or not heteroatom(s), saturated, insaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, or heterocycloalkyl.

20 For example, cephalotaxine esters of the following formulas:

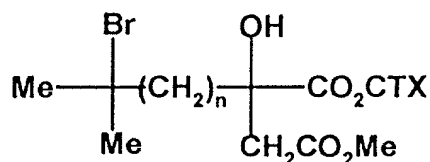




Where  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^8$ ,  $\text{Z}$ ,  $\text{X}$  and  $\text{CTX}$  are as defined above;

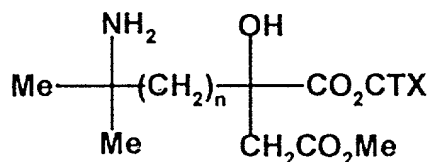
- bromodeoxyharrintonine ( $n = 2$ ) and bromodeoxyhomoharrintonine ( $n = 3$ )

5



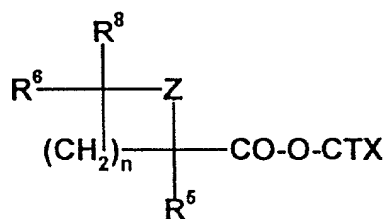
where  $\text{CTX}$  is as defined above;

- aminodeoxyharrintonine ( $n = 2$ ) and aminodeoxyhomoharrintonine ( $n = 3$ )



10 where  $\text{CTX}$  is as defined above;

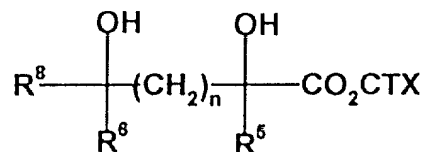
In addition, when the cyclic side-chain of sidechain-bearing cephalotaxane, and/or a salt thereof, presents the following formula:



15 where  $n$ ,  $\text{R}^5$ ,  $\text{R}^6$ ,  $\text{R}^8$ ,  $\text{CTX}$  and  $\text{Z}$  are as defined above,

the said chain is open by treatment with a solution of hydrobromic acid in acetic acid, in an halogenated solvent, preferably dichloromethane, followed by *in situ*

hydrolysis to provide, without isolation of the intermediate, a sidechain-bearing cephalotaxane of the following formula:



5 where n, CTX, R<sup>5</sup>, R<sup>6</sup> et R<sup>8</sup> are defined above.

Acids corresponding to the following formula :



where  $\Omega$  radical is as defined according above;

10 the said formula equivalent to racemic mixture containing compounds of the formulas (+)- $\Omega$ -CO-OH and (-)- $\Omega$ -CO-OH such as (+)- $\Omega$ -CO-OH represents its dextrogyre enantiomer and (-)- $\Omega$ -CO-OH represent its levogyre enantiomer, were obtained

a) by contacting of said racemic mixture or one of its activated form of the  
15 formula



which is as defined above;

the said racemic mixture or said activated form generating respectively:

- 20
- either an anion corresponding to the formula ( $\Omega\text{-CO-O}$ )<sup>-</sup>;
  - or a cation corresponding to the formula ( $\Omega\text{-CO}$ )<sup>+</sup>;

with a pure enantiomeric form of chiral entity, said "resolution agent" symbolized by  $\Delta^*$  (delta stella), having the ability to form:

- 25
- either a stable combination, by covalent bonding;
  - or an easily reversible labil combination, by hydrogen bonding or by hydrophobic interaction;

- or intermediate lability bonding by electrostatic interaction;

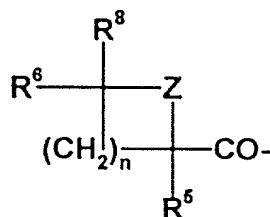
to provide a diastereomeric mixture of  $\Omega$ -CO-O- $\Delta^*$  and de  $\Omega$ -CO- $\Delta^*$ ;

- 5 b) then by physical separation of the mixture of two diastereomers or two complex compounds or more generally of two new entities physically and/or chemically different then obtained;

- 10 c) then by regeneration and finally separation of each one of enantiomers of the generic formula  $\Omega^*$ -CO-OH, where  $\Omega^*$  («oméga stella») represents the generic symbol of the same chiral radical in the either one or the other pure enantiomeric forms corresponding to the following formulas **(+)- $\Omega$ -CO-OH** and **(-)- $\Omega$ -CO-OH** which are as defined above.

- 15 Preferably,  $\Omega$ -CO- is:

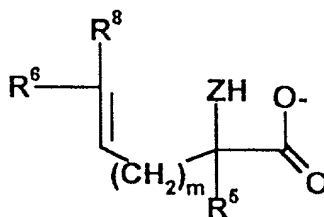
- either a radical corresponding to the following formula:



where n, Z,  $\text{R}^6$ ,  $\text{R}^8$ , and  $\text{R}^5$  are as defined above;

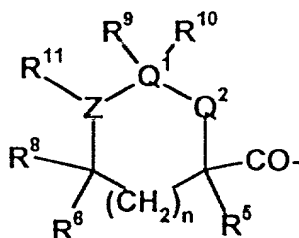
- or a radical corresponding to the following formula:

20



where m, Z,  $\text{R}^6$ ,  $\text{R}^8$ , and  $\text{R}^5$  are as defined above;

- or a radical corresponding to the following formula:



5 where n, R<sup>5</sup>, R<sup>6</sup>, R<sup>8</sup>, Z, Q<sup>2</sup>, Q<sup>1</sup>, R<sup>9</sup>, R<sup>10</sup> and R<sup>11</sup> are as defined above.

The stable combination may be represented by an ester of the following formula  $\Omega$ -CO-O- $\Delta^*$  such as  $\Omega$  and  $\Delta^*$  are as defined above, the said stable combination is obtained by contacting acid with a chiral alcohol corresponding to the formula HO- $\Delta^*$  such as  $\Delta^*$  is as defined above, according the process of invention.

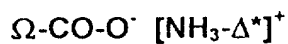
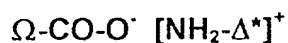
The stable combination may be represented by an amide corresponding to the either one or the other formulas  $\Omega$ -CO-NH- $\Delta^*$  or  $\Omega$ -CO-N- $\Delta^*$  such as  $\Omega$  and  $\Delta^*$  are as defined above, the said stable combination is obtained by contacting acid with primary or secondary chiral amine corresponding to formulas H<sub>2</sub>N- $\Delta^*$  or NN= $\Delta^*$  such as  $\Delta^*$  is as defined above, according the process of the invention.

20 The stable combination may be represented by an thioester of the following formula  $\Omega$ -CO-S- $\Delta^*$  such as  $\Omega$  and  $\Delta^*$  are as defined above, the said stable combination is obtained by contacting acid with a chiral thiol corresponding to the formula HS- $\Delta^*$  such as  $\Delta^*$  is as defined above, according the process invention.

25 Finally, the ionic combination may be represented by a salt just prepared by contacting of acid with a chiral amine corresponding to the either one or the



other of the three following formulas:



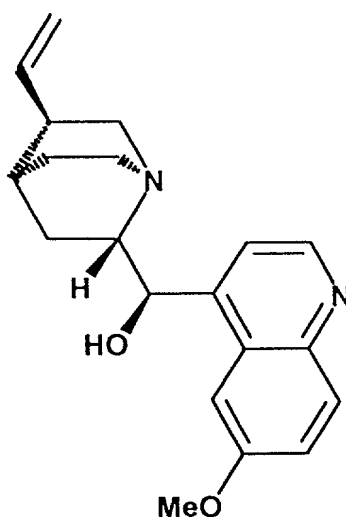
5 where  $\Omega$  and  $\Delta^*$  are as defined above.

The bringing into play of a labil bonding based combination is achieved in the form of chromatography with the help of a chiral stationary phase.

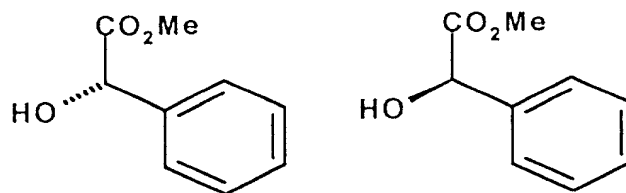
10 The bringing into play of an interatomic or intermolecular labil bonding based combination, within crystalline lattice, is achieved in the form of fractionated crystallization initiated by a chiral precursor.

The chiral alcohol HO- $\Delta^*$  is:

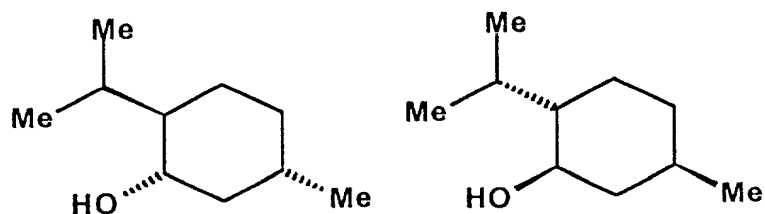
15 - either (-)-quinine corresponding to the following formula:



20  
25  
- or (-)- or (+)-methyl mandelate corresponding to the following formulas:

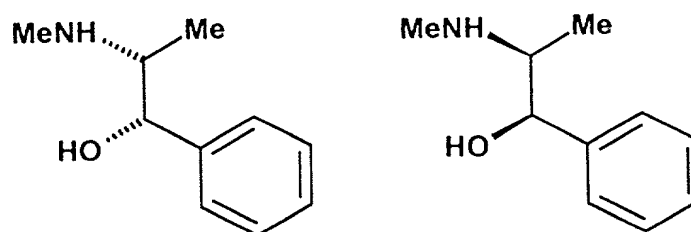


- or (-)- or (+)-menthol corresponding to the following formulas:

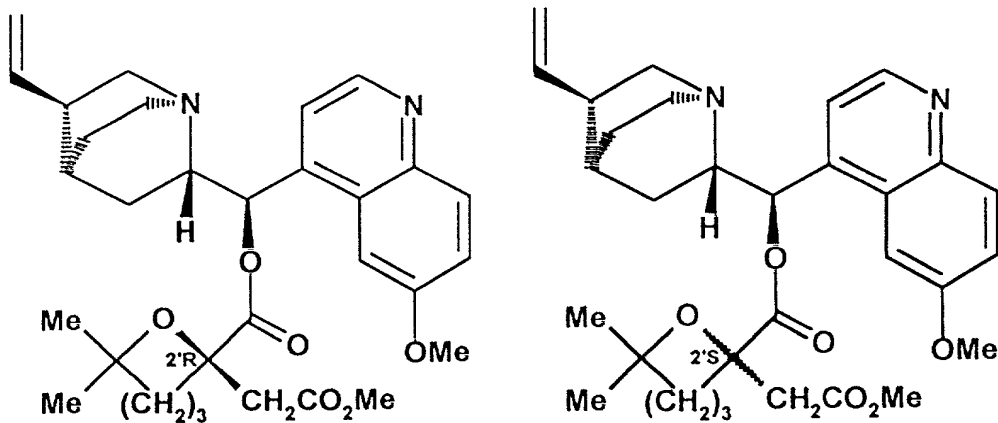


5

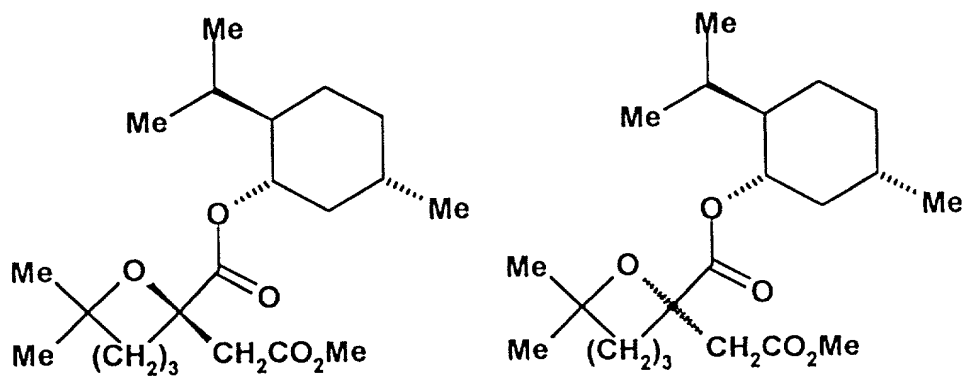
The chiral amine  $H_2N-\Delta^*$  is (-)- or (+)-ephedrine corresponding to the following formulas:



- 10 The present invention concerns the following new compounds:
- the (-)-quinidyl (2'R)-(-)-anhydro-homoharringtonate and the (-)-quinidyl (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:

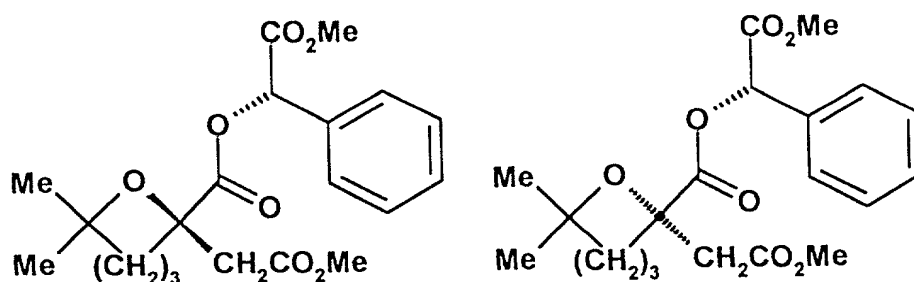


- the (-)-menthyl (2'R)-(-)-anhydro-homoharringtonate and the (-)-menthyl (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:



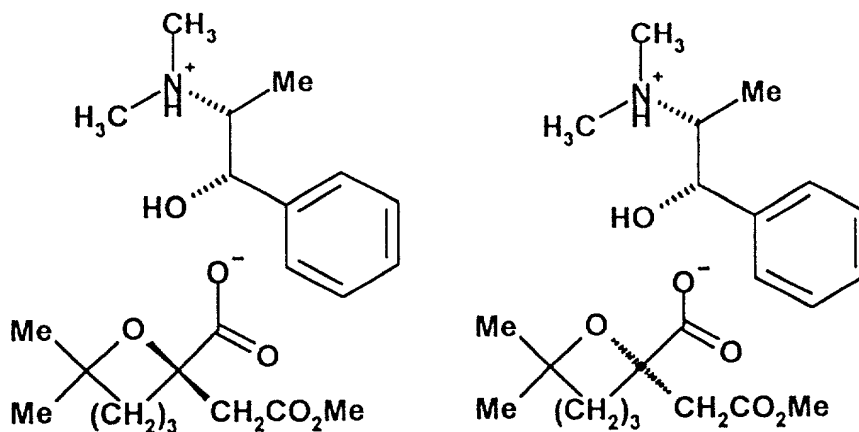
5

the (-)-methylmandelyl (2'R)-(-)-anhydro-homoharringtonate and the (-)-methylmandelyl (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:

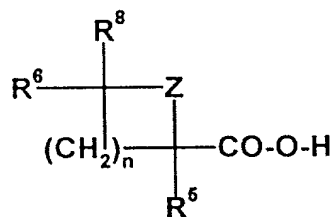


- the (-)-ephedrinium (2'R)-(-)-anhydro-homoharringtonate and the (-)-ephedrinium (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:

5



According the process of invention, when the carboxylic acid is the tertiary heterocycloalcanecarboxylic acid corresponding to the following formula:

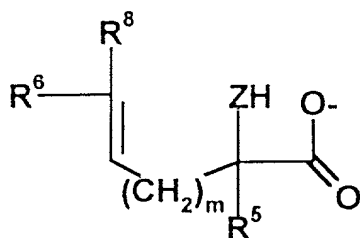


10

where  $n$ ,  $Z$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above, the said acid is obtained by treatment in aprotic or protic solvent, eventually in the presence of cyclization additive and/or dehydrating agent, the said treatment eventually supported with physical carrying of the water formed.

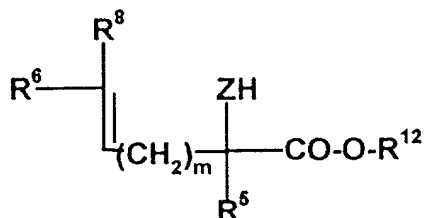
15

- or open tertiary ethylenic acid corresponding to the following formula:



where  $m$ ,  $Z$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above.

- or open tertiary ethylenic acid corresponding to the following formula:



5

where  $m$  is included between 1 and 8,  $Z$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above,  $R^{12}$  is not a CTX radical as defined above and represents  $R^5$  and/or a protective group of acids and/or a chiral group;

then  $R^{12}$  is removed later, either just by saponification, or by hydrogenolysis,  
 10 or more generally by the method of the state of art to remove protective groups of acids.

In the absence of cyclization additive, the reaction of cyclization just take place by heating.

15

Preferably, the cyclization additive is a protic acid such as sulfonic or formic acid, or an aprotic acid, included in immobilized form.

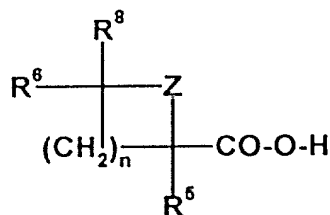
In the step of preparation of the acid described above,  $Z$  is an oxygen atom.

20

The aim of the present invention is also the preparation of the following new compounds:

- the tertiary heterocycloalcane carboxylic acid, included its salts and each one

of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



where  $n$ ,  $Z$ ,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined above, and  $\text{R}^5$  is not hydrogen;

except for compounds where  $Z$  is oxygen atom and,

1°)  $n = 0$  and  $\text{R}^5$  is not  $-\text{CH}_2\text{CO}_2\text{H}$  or  $-\text{CH}_2\text{CO}_2\text{CH}_3$  radical;

2°)  $n = 0$  and  $\text{R}^5$  is  $-\text{CH}_2\text{CO}_2\text{H}$  or  $-\text{CH}_2\text{CO}_2\text{CH}_3$  radical, and  $\text{R}^6 = \text{R}^8 = \text{methyl}$  or  $-\text{CH}_2\text{CO}_2\text{H}$  or  $-\text{CH}_2\text{CO}_2\text{CH}_3$  radical;

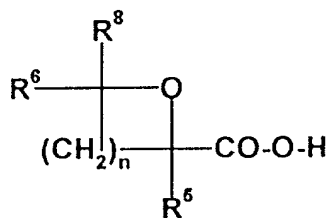
3°)  $n = 2$  and simultaneously  $\text{R}^6 = \text{R}^8 = \text{methyl}$ , and  $\text{R}^5 = \text{OMe}$  or hydroxyl ;

4°)  $n = 2$  and simultaneously  $\text{R}^6 = \text{R}^8 = \text{methyl}$ , and  $\text{R}^5$  is  $-\text{CH}_2\text{CO}_2\text{H}$  or  $-\text{CH}_2\text{CO}_2\text{CH}_3$  radical or methyl;

5°)  $n = 3$  and simultaneously  $\text{R}^6$  is hydroxyl, and  $\text{R}^8$  is methyl, and  $\text{R}^5$  is  $-\text{CH}_2\text{CO}_2\text{CH}_3$  radical;

6°)  $n = 3$  and simultaneously  $\text{R}^6 = \text{R}^8 = \text{methyl}$  and  $\text{R}^5 = \text{OH}$  or methyl or ethyl.

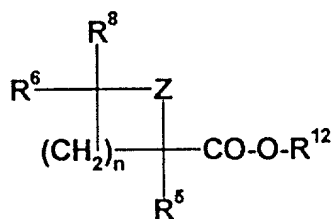
- the tertiary oxacycloalcanecarboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



where n is included between 0 and 8, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined above, but are not hydrogen simultaneously.

except for compounds corresponding to the exceptions 1 to 6 defined above.

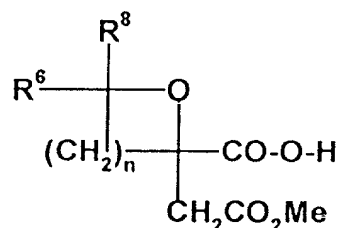
- 5 - the tertiary heterocycloalkane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



- 10 where n is included between 0 and 8, Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined above, R<sup>5</sup> is not hydrogen, and R<sup>12</sup> is not a CTX radical defined above;

except for compounds where Z is oxygen atom and, corresponding to the exceptions 1 to 6 defined above.

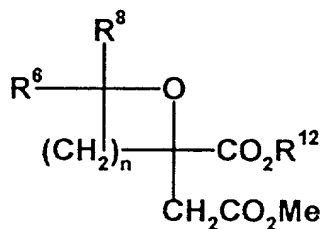
- 15 - the tertiary oxacycloalkane carboxylic hemiester, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



where n is included between 0 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined above.

except for compounds corresponding to the exceptions 1 to 6 defined above.

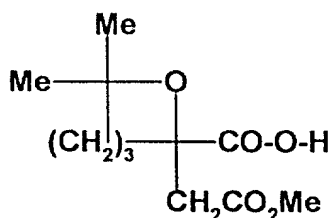
- 5 - the tertiary oxacycloalcane carboxylic hemiester, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



where n is included between 0 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined above,  $\text{R}^{12}$  is an hydrocarbon radical different from CTX as defined above.

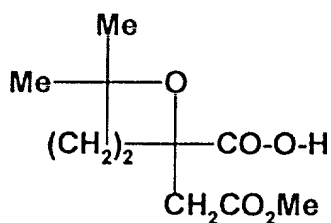
except for compounds corresponding to the exceptions 1 to 6 defined above.

- 15 - the tertiary oxacycloalcane carboxylic hemiester or anhydro-homoharringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



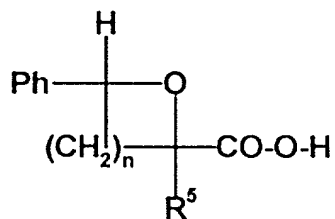


- the tertiary oxacycloalcane carboxylic hemiester or anhydro-harringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



- the tertiary oxacycloalcane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

10

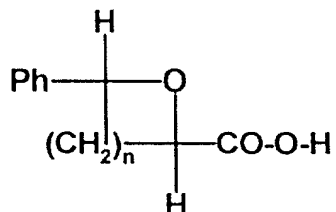


where  $n$  and  $\text{R}^5$  are as defined above,

except for compounds corresponding to the exceptions 1 to 6 defined above.

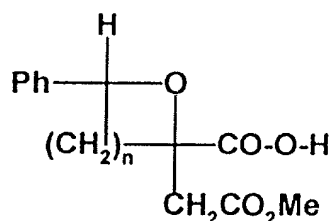
- the tertiary oxacycloalcane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

15



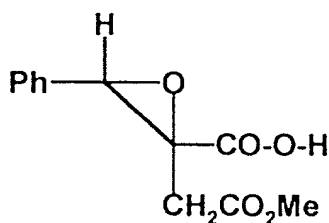
where  $n$  is included between 1 and 8.

- the tertiary oxacycloalcane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

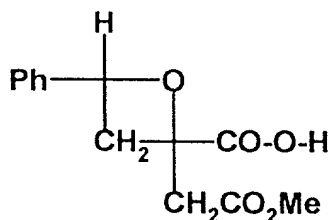


where n is included between 0 and 8.

- the tertiary oxacycloalcane carboxylic acid or oxanhydroneoharringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

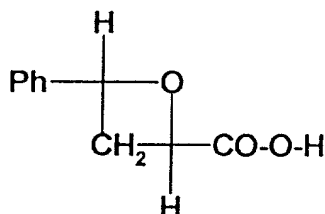


- the tertiary oxacycloalcane carboxylic acid or oxanhydro-neohomoharringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

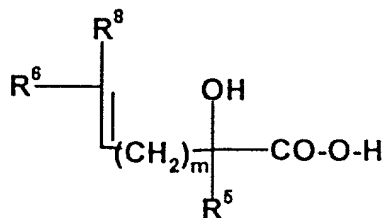


- the tertiary oxacycloalcanecarboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

5

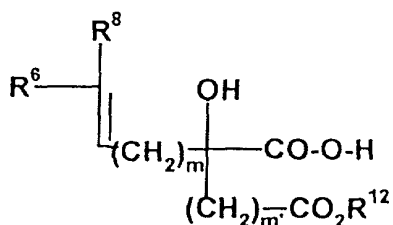


- the tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



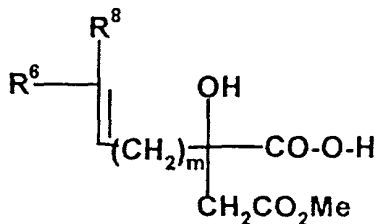
10 where  $m$  is included between 1 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined above, but are not hydrogen simultaneously, and  $\text{R}^5$  is not hydrogen or heteroatom.

15 - the tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



where  $m$ ,  $\text{R}^6$ ,  $\text{R}^8$  and  $\text{R}^{12}$  are as defined above, and  $m'$  is included between 1 and 8.

- 5 - the tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

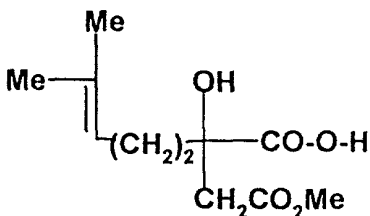


where  $m$  is included between 1 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined above but are not hydrogen.

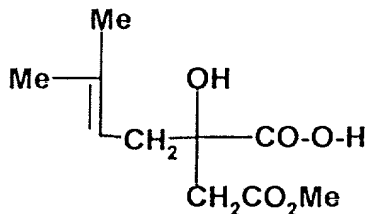
10

- the tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

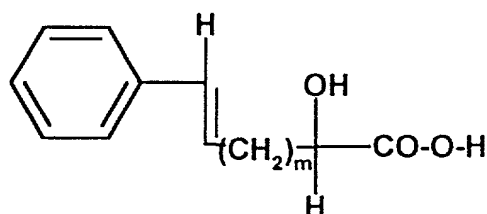
15



- the tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



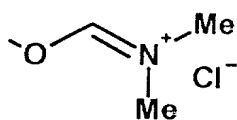
- the tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



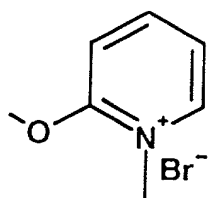
where m is included between 1 to 8, preferably m = 1.

- the anhydrides of acid of the general formula  $\Omega$ -CO-O-CO- $\Omega$  where  $\Omega$  is as defined above.
- the mixed anhydrides of acid of the general formula  $\Omega$ -CO-A where A is a radical selected from the following substituents:
- méthoxyformyloxy of formula MeOCOO-,
  - trifluoroacétyloxy of formula CF<sub>3</sub>COO-,
  - alkylsulfonyloxy of formula RSO<sub>3</sub>-,
  - phosphoxy of formula (RO)<sub>2</sub>PO-,
  - halophosphoxy of formula ROP(Cl)O-,
  - trialkylsilyloxy of formula R<sub>3</sub>SiO-,
- formulas wherein R is alkyl.

diméthyl-formamidinium chloride of formula



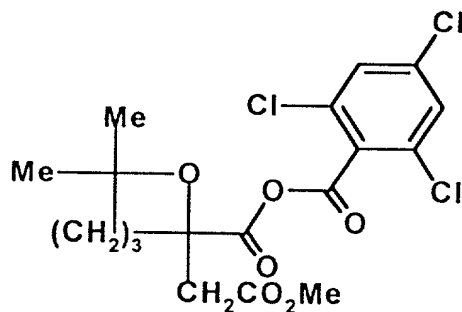
acyloxy-pyridinium bromide of formula



5

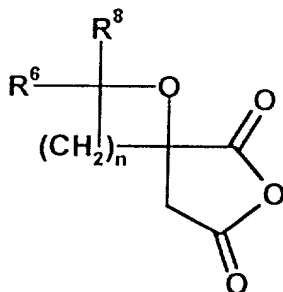
and 2,4,6-trichlorobenzoyloxy.

- the mixed anhydride corresponding to the following formula:



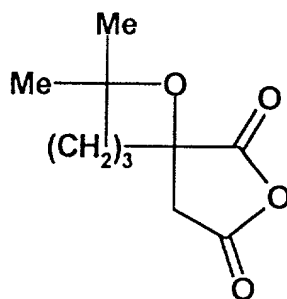
- 10 - the acid chlorides defined above, corresponding to the general formula  $\Omega$ -CO-X, where X is halogen.

- the cyclic anhydrides corresponding to the following formula:



where  $n$ ,  $R^6$  and  $R^8$  are as defined above.

- the cyclic anhydride corresponding to the following formula:



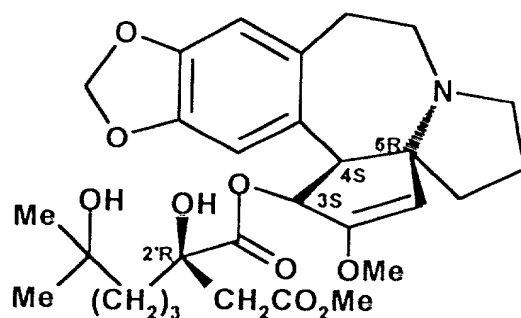
5

In the process according to the invention, the sidechain-bearing cephalotaxane was purified like a salt by chromatography using a hydrophobic reversed-phase like stationary phase, and a mobile phase without organic solvent like a solution adjusted to a pH 2 to 4.5 with a buffer prepared with an acid and an alkaline or ammonium salt and one or several additive like attenuator of silanol effect, the said cephalotaxine salt was generated from mineral acid under the form of chlorohydrate, sulfate, phosphate, nitrate, perchlorate, or from organic acid under the form of tartrate, malate, citrate or lactate.

10

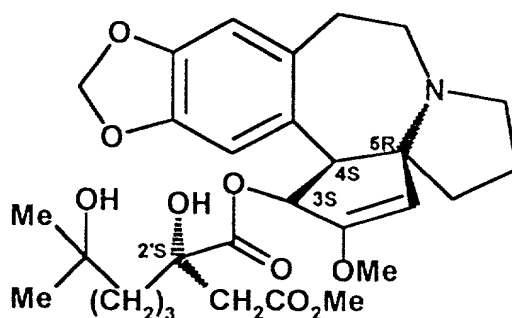
15

In the process according to the invention, the sidechain-bearing cephalotaxane was purified by a step of chromatographic purification of a natural or semi-synthetic or synthetic homoharringtonine as a pharmaceutical use corresponding to the following formula:



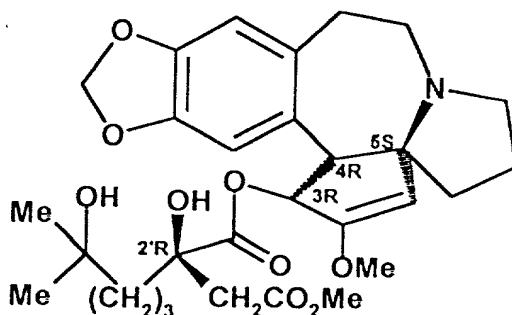
to remove the undesired related impurity named 2'-épi-homoharringtonine  
 5 resulting:

a) either from a semi-synthetic process with introduction of a synthetic  
 homoharringtonic acid of inadequate enantiomeric purity, the generated  
 impurity showing the absolute configuration corresponding to the following  
 10 formula:



b) or from the biosynthetic process in the plant, where a cephalotaxine with  
 inadequate enantiomeric purity was introduced, or in the form of artefact by  
 15 partial racemization of the cephalotaxine moiety, the generated impurity  
 showing strictly identical chromatographic properties with a non-chiral system,  
 with an absolute configuration opposite to the one above (enantiomer) and  
 corresponding to the following formula:





especially making use of one of the following chromatographic systems:

A) Stationary phase: alkyl- or phenyl- or alkylphenyl- ou phenylalkyl-silane, preferably *n*-octadecylsilane,

5 B) Mobile phase: water-tetrahydrofurane, water-methanol, water-acetonitrile or buffer pH 2 to 6.5 in replacement of water, or all other mobile phase with equivalent selectivity,

10 This process of purification and chromatographic control of a natural or semi-synthetic or synthetic homoharringtonine, allows to offset the double insufficiency of enantiomeric purity of the semi-precursors, both on the sidechain precursor (said homoharringtonic acid) and cephalotaxine, the two said-precursors are each independently generated by total synthesis or semi-synthetic process or natural process within of the plant (biosynthesis), in fact the withdrawal of the non natural enantiomer of homoharringtonine showing an  
15 opposite absolute configuration, by using a chiral stationary phase with preparative scale.

20 The present invention is illustrated without implied limitation by the following schemes.

**Scheme 1** in annex gives the definition and the formulas of main harringtonines.

**Scheme 2** recapitulates process of preparation of harringtonines of prior art.

25 **Scheme 3** gives the sequence of synthesis of homoharringtonine corresponding to the Example 25, where A represents a 2,4,6-trichloro-phényl group, R represents a methyl and R' represents a cephalotaxyl moiety.

**Scheme 4** represents a variant of the process according to the invention, more exactly the semi-synthesis of harringtonines *via* oxacycloalcalne carboxylic

acids. The substitutes  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R$ ,  $A$ ,  $CTX$ ,  $X$  and the letters  $n$  et  $m$  referenced in this scheme are defined in the description.

The following definitions applies for the whole present document.

5

Alkaloids: *natural* substances present in the *vegetal* kingdom allowing at least a cyclic or acyclic *basic* nitrogen (allowed extensions: animal kingdom, primary amine; refused extensions: amidic nitrogen, because non basic, ex: taxanes) and showing frequently pronounced pharmacological properties.

10

Basic or free Alkaloid: alkaloid showing a tertiary amine in a non-ionized form generally existing at alkaline pH and soluble in aprotic organic solvents.

15

Salt of alkaloid or just «salt»: ionized form of alkaloid with amine function showing a positive formal charge and a negative counter-ion, actually more soluble in water and protic solvents and less soluble in aprotic solvents.

20

Cephalotaxanes 1 (see **scheme 1**): this generic term indicates the basic framework, showing diverse oxygenated substitutes (aliphatic or aromatic ether, free or esterified alcohol, enol and/or free or substituted phenol, bridged ether, more generally all substitute usually founded at natural state for this kind of compounds).

25

Cephalotaxines 2: this generic term indicates cephalotaxanes possibly showing at least one of the substitutes described above, a sidechain excepted.

Cephalotaxine 2: a cephalotaxine in majority present in the genus *Cephalotaxus*

30

Cephalotaxoids: this generic term indicates a non-natural cephalotaxine.

Harringtonines: this generic term indicates cephalotaxane showing at least an alcohol group, a phenol or an enol, esterified by a sidechain and possibly by one of the substitutes described above.

Harringtonine (the): one of the main alkaloids bearing a sidechain in position 3.

Harringtoids: this generic term indicates a non-natural harringtonine, where  
5 sidechain is an ester radical showing at least 3 carbon atoms.

Sidechain of the harringtoids: this generic term indicates an ester formed  
between one of the hydroxyl group and a carboxylic acid showing at least 3  
carbon atoms allowing usually a tertiary alcohol tertiary in  $\alpha$  position and an  
10 hydrophobe substitute in  $\omega$  position relatively to the carboxyl group.

Adaptation of the additive et subtractive empirical nomenclature suitable for  
céphalotaxanes.

15 Prefixes of common nomenclature are usually used in the literature to indicate  
structural variations of the sidechain of harringtonines (see Examples in the  
table of scheme 1). The sidechain of reference is this of harringtonine showed  
in formula 3b,  $n = 2$ ,  $R = H$ ,  $R^7 = H$ ,  $R^6 = 2$ -hydroxyisopropyl.

20 *Homo*: 1 extra carbon.

*Bishomo*: 2 extra carbons.

*Nor*: a sidechain with 1 carbon less.

*Iso*: a sidechain with methylene bearing an hydroxyl group at the place of  
juxtaterminal carbon.

25 *Deoxy*: the hydroxyl group of juxtaterminal carbon is replaced par hydrogen.

*Anhydro*: the two tertiary hydroxyl groups lose a molecule of water to give the  
corresponding saturated oxygenated heterocycle.

*neo*:  $R^6$  is a phenyl group at the place of 2-hydroxyisopropyl.

30 HPLC: High-Performance Liquid Chromatography.

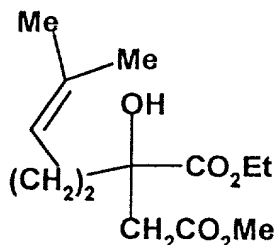
NMR: Nuclear Magnetic Resonance.

The following examples, which are given without implied limitation, illustrates the present invention.

5

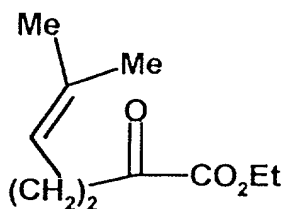
### Example 1

#### Preparation of ethyl 2-methoxycarbonylmethyl-2-hydroxy-6-methylhept-5-enoate or ethyl 6-desoxy-5,6-dehydrohomoharringtonate:



#### 10 **1°) Preparation of the intermediate oxalate**

- 5-Bromo-2-methyl-pent-2-ene (15.6 g, 95.6 mmol) was added dropwise to a stirred mixture of magnesium (2.32 g, 95.5 mmol)(activated with further crystal of iodine) in anhydrous tetrahydrofuran (75 ml). The onset of the reaction is accompanied with a vigorous overheating and refluxing of the reaction mixture.
- 15 The reflux was maintained until most of magnesium had reacted and the reaction mixture was diluted with anhydrous tetrahydrofuran (150 ml). To a stirred mixture of diethyl oxalate (10.8 ml, 80 mmol) in anhydrous tetrahydrofuran (75 ml) was added at  $-78^{\circ}\text{C}$  the resulting Grignard reagent over a period of 20 minutes. The stirring was maintained at  $-78^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 30
- 20 minutes and then the temperature was raised to  $-10^{\circ}\text{C}$  over a period of 1.5 hours. The mixture was quenched with 15% ammonium chloride solution (300 ml). The separated organic layer was washed with 15% ammonium chloride solution (300 ml) and evaporated to dryness. The aqueous layer was extracted with ether (2 x 300 ml). The organic layers were combined with the concentrate
- 25 and washed with brine (300 ml), dried over magnesium sulfate and evaporated to dryness. The crude product, after purification with a bulb-to-bulb distillation apparatus, afforded colorless oil (10.3g, 70 %). The intermediate  $\alpha$ -cetoester showed the following characteristics:



IR (ATR) ( $\text{cm}^{-1}$ ): 2790; 2916; 1725; 1068.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

5.08 (1H, t,  $J = 7.2$ , HC=); 4.32 (2H, q,  $J = 7.1$ ,  $\text{OCH}_2$ ); 2.86 (2H, t,  $J = 7.2$ ,  $\text{CH}_2\text{CO}$ ); 2.32 (2H, q,  $J = 7.2$ ,  $\text{CH}_2\text{-C=}$ ); 1.68 (3H, s,  $\text{CH}_3$ ); 1.62 (3H, s,  $\text{CH}_3$ ); 1.37 (3H, t,  $J = 7.1$ ,  $\text{OCH}_2\text{CH}_3$ ).

## 2°) Preparation of the title product

Anhydrous methyl acetate (0.6 ml, 7.5 mmol) was added to a stirred commercial solution of lithium bis-(trimethylsilylamide) 1M in tetrahydrofuran (7.5 ml, 7.5 mmol) at  $-78^\circ\text{C}$  over a period of 1 minute and this was allowed the reaction to proceed at  $-78 \pm 5^\circ\text{C}$  for 20 minutes. To a stirred mixture of ethyl 2-oxo-6-methylhept-5-enoate prepared above (480 mg, 2.6 mmol) in anhydrous tetrahydrofuran (10 ml) at  $-78^\circ\text{C}$  was added the lithium enolate over a period of 5 minutes, and the resulting mixture was stirred at  $-78 \pm 5^\circ\text{C}$  for 30 minutes. After monitoring in CCM, the freezing bath was removed and the mixture was quenched with 15% ammonium chloride solution (10 ml). The separated organic layer was washed with 15% ammonium chloride solution (10 ml) and evaporated to dryness. The aqueous layers were extracted with ether (2 x 10 ml). The organic layers were combined with the concentrate and washed with brine (10 ml), dried over magnesium sulfate and evaporated to dryness. The resulting crude product (1.13 g) was purified by column chromatography (cyclohexane / ethyl acetate (95:5), silica (15-40  $\mu\text{m}$ ) 38 g) to provide a colorless oil (482 mg, 72%). The product thus obtained showed the following characteristics:

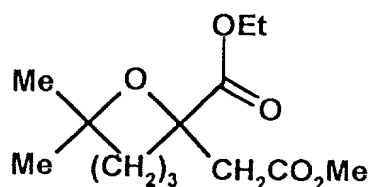
IR (ATR) ( $\text{cm}^{-1}$ ): 3508; 2969; 2919; 1732; 1438; 1193.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

5.05 (1H, t,  $J = 7.1$ , HC=); 4.27 (2H, q,  $J = 7.1$ ,  $\text{OCH}_2$ ); 3.70 (1H, s, OH); 3.68 (3H, s,  $\text{OCH}_3$ ); 2.92 and 2.70 (2H, 2d,  $J_{\text{AB}} = 16.1$ ,  $\text{CH}_2\text{CO}_2$ ); 2.12 (1H, m); 1.88 (1H, m); 1.72 (2H, m); 1.67 (3H, s,  $\text{CH}_3$ ); 1.58 (3H, s,  $\text{CH}_3$ ); 1.31 (3H, t,  $J = 7.1$ ,  $\text{OCH}_2\text{CH}_3$ ).

Example 2

Preparation of ethyl 2-methoxycarbonylmethyl-2-hydroxy-6,6-dimethyl-2-tetrahydropyrane carboxylate or ethyl anhydrohomoharringtonate :

**1°) Method A**

p-Toluenesulfonic acid (2,06 g, 10,8 mmol) was added to a stirred solution of ethylenic ester resulting from Example 1 (2.8 g, 10,8 mmol) in toluene (30 ml) and the resulting mixture was stirred at 65°C for 5 hours. After cooling at room temperature, the mixture was hydrolyzed with saturated sodium hydrogen carbonate solution. The aqueous layer was extracted with ether (3 x 50 ml), and the organic layers were combined, washed with brine (100 ml), dried over magnesium sulfate and evaporated to dryness. The resulting crude product (2.8 g) was purified by column chromatography (cyclohexane / ether (95:5), silica (15–40  $\mu$ m) 110 g) to provide a colorless oil (1.94 g, 69%). The product thus obtained showed the following characteristics:

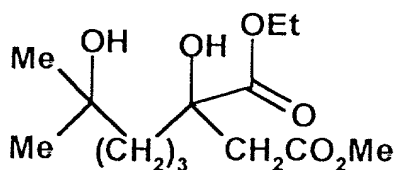
<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) ( $\delta$  ppm, J Hz):

4.21 (2H, m, OCH<sub>2</sub>CH<sub>3</sub>); 3.64 (3H, s, OCH<sub>3</sub>); 2.85 and 2.60 (2H, 2d, J<sub>AB</sub> = 14.0, CH<sub>2</sub>CO<sub>2</sub>); 2.30 (1H, dt, J = 13.3 and 3.7); 1.87 (1H, qt, J = 13.8 and 3.6); 1.62 (1H, m); 1.51 (2H, m); 1.43 (1H, m); 1.31 (3H, t, J = 7.1, OCH<sub>2</sub>CH<sub>3</sub>); 1.22 (3H, s, CH<sub>3</sub>); 1.13 (3H, s, CH<sub>3</sub>).

**2°) Method B**

To a stirred solution of ethylenic ester resulting from Example 1 (50 mg, 0.19 mmol) in methanol (30 ml) was added hydrochloric acid 1N (0.5 ml) and the resulting mixture was stirred at 65°C for 15 hours. After dilution with dichloromethane, the organic layer was dried over magnesium sulfate and evaporated to dryness. The resulting crude product (32 mg) was purified by column chromatography (dichloromethane, then dichloromethane / methanol

(9:1), silica (15–40  $\mu\text{m}$ ) 2.2 g) to provide the expected intermediate diol (20 mg, 37%). The product thus obtained showed the following characteristics:



5 IR (ATR) ( $\text{cm}^{-1}$ ): 3490; 2966; 1731; 1193; 1177; 1152.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

4.28 (2H, q, J = 7.2,  $\text{OCH}_2$ ); 3.75 (1H, s, OH); 3.68 (3H, s,  $\text{OCH}_3$ ); 2.93 and  
 2.69 (2H, 2d,  $J_{\text{AB}} = 16.2$ ,  $\text{CH}_2\text{CO}_2$ ); 1.70 (2H, m); 1.53 (1H, m); 1.44 (1H, m);  
 10 1.30 (3H, t, J = 7.1,  $\text{OCH}_2\text{CH}_3$ ); 1.20 (3H, s,  $\text{CH}_3$ ); 1.19 (3H, s,  $\text{CH}_3$ ).

To a stirred solution of diol prepared above (19 mg, 0.069 mmol) in 1,2-dichloroethane (1.4 ml) was added anhydrous zinc chloride (10 mg, 0.069 mmol) and the resulting mixture was stirred at 80°C for 1.5 hours. After cooling  
 15 at ambient temperature, the mixture was washed with water, then with brine, and the aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford cyclic diester (7 mg, 40%). The product thus obtained showed identical characteristics to this obtained with method A.

20

### 3°) Method C

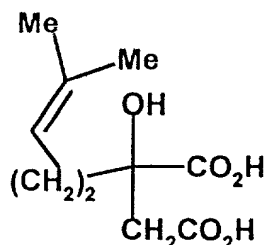
A solution of ethylenic ester resulting from Example 1 (400 mg, 1.55 mmol) in a mixture of formic acid (4 ml) and water (4 ml) was stirred at 50°C for 15 hours. After removal of formic acid *in vacuo*, the residue was treated with 5% sodium  
 25 hydrogen carbonate solution. The aqueous layer was extracted three times with dichloromethane then the combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (375 mg) was purified by column chromatography (dichloromethane, then dichloromethane / methanol (98:2), silica (15–40  $\mu\text{m}$ ) 16 g) to provide a colorless oil (235 mg,  
 30 55%). The product thus obtained showed identical characteristics to this obtained with method A. The cyclisation of the diol thus obtained with zinc

chloride, like Example 2 method B above, afforded cyclic diester showing identical characteristics to this obtained with method A.

5

### Example 3

#### Preparation of 2-carboxymethyl-2-hydroxy-6-methylhept-5-enoic acid or O-demethyl-6-desoxy-5,6-dehydrohomoharringtonic acid:



- 10 A mixture of potassium hydroxide (14.2 g, 252 mmol) in water (170 ml) was added to a stirred solution of ethylenic ester resulting from Example 1 (10.95 g, 42 mmol) in methanol (300 ml) and the resulting mixture was stirred at reflux for 1.5 hours. After cooling at room temperature, and removal of methanol *in vacuo*, the residue was treated with water (10 ml) and the resulting aqueous layer was extracted with ether (250 ml). After acidification (pH 1) with 10% hydrochloric acid, the aqueous layer was extracted with ether (3 × 250 ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a white solid (8.66 g, 95%). The crude product thus obtained showed the following characteristics:

20

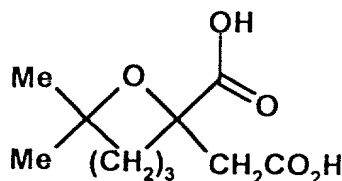
IR (ATR) (cm<sup>-1</sup>): 3500; 3019; 2966; 2931; 1716; 1691; 1656; 1219; 1199; 1111.

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

- 5.06 (1H, t, J = 6.9, HC=); 3.04 and 2.78 (2H, 2d, J<sub>AB</sub> = 17.1, CH<sub>2</sub>CO<sub>2</sub>); 2.25 - 1.20 (4H, m, 2 × CH<sub>2</sub>); 1.67 (3H, s, CH<sub>3</sub>); 1.60 (3H, s, CH<sub>3</sub>).

25



Example 4Preparation of 2-carboxymethyl-6,6-dimethyl-2-tetrahydropyranecarboxylic acid or O-demethylanhydrohomoharringtonic acid:

5

**1°) Method A**

A mixture of potassium hydroxide (4.2 g, 75 mmol) in water (45 ml) was added to a stirred solution of cyclic diester resulting from Example 2 (1.94 g, 7.5 mmol) in ethanol (75 ml) and the resulting mixture was stirred at reflux for 5 hours. After cooling at room temperature, and removal of ethanol *in vacuo*, the residue was treated with water (10 ml) and the resulting aqueous layer was extracted with ether (2 × 50 ml). After acidification with hydrochloric acid 2N (35 ml), the aqueous layer was saturated with sodium chloride then was extracted with ether (3 × 50 ml). The combined organic layers were washed with brine (2 × 100 ml) dried over magnesium sulfate and evaporated to dryness to afford a pale yellow oil (1.66 g, 98%). The crude product thus obtained showed the following characteristics:

20

IR (ATR) (cm<sup>-1</sup>): 2974; 2941; 1709; 1215.

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

3.01 and 2.95 (2H, 2d, J<sub>AB</sub> = 16.1, CH<sub>2</sub>CO<sub>2</sub>); 1.89 (1H, m); 1.75 (2H, m, CH<sub>2</sub>); 1.58 (3H, m); 1.31 (6H, s, 2 × CH<sub>3</sub>).

**2°) Method B**

To a stirred solution of ethylenic diacid resulting from Example 3 (50 mg, 23 mmol) in anhydrous toluene (500 μl) was added zinc chloride (6 mg, 0.04 mmol) and the resulting mixture was stirred at 80°C for 15 hours. After cooling at room temperature, the mixture was hydrolyzed with 10% hydrochloric acid, and the resulting aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate and

evaporated to dryness to afford a pale yellow solid (38 mg, 76%). The crude product thus obtained showed identical characteristics to this obtained with method A.

5

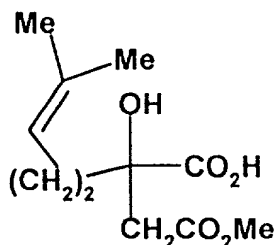
### 3°) Method C

A solution of ethylenic diacid resulting from Example 3 (50 mg, 0.23 mmol) in a mixture of formic acid (500  $\mu$ l) and water (500  $\mu$ l) was stirred at 60°C for 3 hours. After cooling at room temperature and removal of formic acid *in vacuo*, the residue was treated with ethyl acetate. The resulting organic layer was washed with 10% hydrochloric acid and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a pale yellow solid (50 mg, 100%). The crude product thus obtained showed identical characteristics to this obtained with method A.

15

### Example 5

20 Preparation of 2-methoxycarbonylmethyl-2-hydroxy-6-methylhept-5-enoic acid or 6-desoxy-5,6-dehydrohomoharringtonic acid:



A mixture of ethylenic diacid resulting from Example 3 (500 mg, 2.3 mmol) and a commercial solution of boron trifluoride-methanol complex in methanol (4.5 ml, BF<sub>3</sub> 12% w/w) was stirred at 18  $\pm$  5°C for 16 hours. After careful addition of the reaction mixture at saturated sodium hydrogen carbonate solution (50 ml), the resulting aqueous layer was washed with ether (50 ml), acidified (pH 1) with hydrochloric acid 2N (0.5 ml) and extracted with ether (3  $\times$  50 ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a viscous yellow oil (310 mg, 58%). The crude product thus obtained showed the following characteristics:

30

IR (ATR) ( $\text{cm}^{-1}$ ): 3483; 2954; 1731; 1197; 1173.

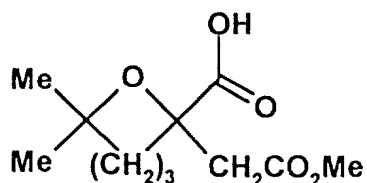
$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

- 5 5.06 (1H, m, HC=); 4.12 (2H, br.s,  $\text{CO}_2\text{H}$  + OH); 3.73 (3H, s,  $\text{OCH}_3$ ); 2.99 and 2.74 (2H, 2d,  $J_{\text{AB}} = 16.7$ ,  $\underline{\text{CH}_2\text{CO}_2}$ ); 2.16 (1H, m); 1.98 (1H, m); 1.85 - 1.60 (4H, m); 1.67 (3H, s,  $\text{CH}_3$ ); 1.60 (3H, s,  $\text{CH}_3$ ).

10

### Example 6

Preparation of 2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydropyranecarboxylic acid or anhydrohomoharringtonic acid:



#### 15 $1^\circ$ ) Preparation from cyclic diacid

- A mixture of cyclic diacid resulting from Example 4 (1.6 mg, 7.4 mmol) and a commercial solution of boron trifluoride-methanol complex in methanol (15.5 ml,  $\text{BF}_3$  12% w/w) was stirred at  $18 \pm 5^\circ\text{C}$  for 15 hours. After careful addition of the reaction mixture at saturated sodium hydrogen carbonate solution (50 ml), the resulting aqueous layer was washed with ether ( $2 \times 50$  ml) (to see annex preparation below), acidified (pH 1) with hydrochloric acid 2N (15 ml) and extracted with ether ( $3 \times 75$  ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a yellow oil (1.17 g, 69%). The crude product thus obtained showed the following characteristics:

25

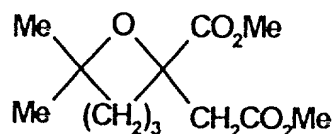
IR (ATR) ( $\text{cm}^{-1}$ ): 2974; 2951; 1740; 1718; 1437.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

- 3.70 (3H, s,  $\text{OCH}_3$ ); 3.03 and 2.98 (2H, 2d,  $J_{\text{AB}} = 16.1$ ,  $\underline{\text{CH}_2\text{CO}_2}$ ); 1.82 (1H, m);  
30 1.74 (3H, m); 1.62 (1H, m); 1.48 (1H, m); 1.31 (3H, s,  $\text{CH}_3$ ); 1.26 (3H, s,  $\text{CH}_3$ ).

## Annex preparations:

## a) Obtaining of diester

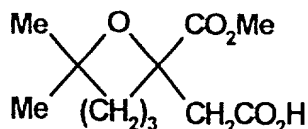


The combined organic layers above-mentioned was dried over magnesium sulfate and evaporated to dryness to afford a mixture of diester and monoester (396 mg). After treatment of this mixture with saturated sodium hydrogen carbonate solution, the aqueous layer was extracted with ether, and the resulting organic layer was dried over magnesium sulfate and evaporated to dryness to afford an oil (292 mg, 17%). The crude product thus obtained showed the following characteristics:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

3.75 (3H, s,  $\text{OCH}_3$ ); 3.65 (3H, s,  $\text{OCH}_3$ ); 2.85 and 2.61 (2H, 2d,  $J_{\text{AB}} = 14.1$ ,  $\text{CH}_2\text{CO}_2$ ); 1.85 (1H, m); 1.62 (1H, m); 1.50 (2H, m); 1.43 (1H, m); 1.21 (3H, s,  $\text{CH}_3$ ); 1.11 (3H, s,  $\text{CH}_3$ ).

## b) Obtaining of regio-hemiester by mono saponification of diester above-mentioned



To a stirred solution of cyclic diester above-mentioned (285mg, 1.17 mmol) in methanol (11 ml) was added a mixture of potassium hydroxide (654 mg, 11.7 mmol) in water (7 ml) and the resulting mixture was stirred at room temperature for 30 minutes. After removal of methanol *in vacuo*, the residue was treated with water (7 ml) and the resulting aqueous layer was extracted three times with ether. After acidification (pH1) with 10% hydrochloric acid solution, the aqueous layer was extracted with. The combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (236 mg) was purified by column chromatography (dichloromethane / methanol

(95:5), silica (15–40  $\mu\text{m}$ ) 6.5 g) to provide a pale yellow solid (220 mg, 82%). The product thus obtained showed the following characteristics:

IR (KBR) ( $\text{cm}^{-1}$ ): 3421; 2960; 2929; 1744; 1705; 1209.

5

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

3.76 (3H, s,  $\text{OCH}_3$ ); 2.76 and 2.67 (2H, 2d,  $J_{\text{AB}} = 15.3$ ,  $\text{CH}_2\text{CO}_2$ ); 2.36 (1H, m,  $J_{\text{AB}} = 13.7$ ,  $J_{3-4} = 3.5$ ,  $J_{3-5} = 1.2$ , H-3<sub>eq</sub>); 1.85 (1H, m,  $J_{\text{AB}} \sim J_{\text{ax-ax}} = 14.0$ ,  $J_{\text{ax-eq}} = 3.7$ , H-4<sub>ax</sub>); 1.67 (1H, m,  $J_{\text{AB}} = 14.1$ ,  $J_{4-3,5} = 3.9$ , H-4<sub>eq</sub>); 1.59 (1H, m,  $J_{\text{AB}} = 13.4$ ,  $J_{5-4} = 3.6$ ,  $J_{5-3} = 1.0$ , H-5<sub>eq</sub>); 1.49 (1H, m,  $J_{\text{AB}} \sim J_{\text{ax-ax}} = 13.2$ ,  $J_{\text{ax-eq}} = 4.0$ , H-3<sub>ax</sub>); 1.42 (1H, m,  $J_{\text{AB}} \sim J_{\text{ax-ax}} = 13.2$ ,  $J_{\text{ax-eq}} = 4.5$ , H-5<sub>ax</sub>); 1.33 (3H, s,  $\text{CH}_3$ ); 1.16 (3H, s,  $\text{CH}_3$ ).

10

## 2°) Preparation from ethylenic hemiester

15 To a stirred solution of ethylenic hemiester resulting from Example 5 (4.6 g, 20 mmol) in toluene (125 ml) was added p-toluenesulfonic acid (3.8 g, 20 mmol) and the resulting mixture was stirred at 65°C for 5 hours. After cooling at room temperature, the mixture was hydrolyzed with saturated sodium hydrogen carbonate solution (100 ml). The aqueous layer was washed with ether (2 x 100 ml) and the organic layers were discarded (to eliminate resulting diester of the reaction). After acidification (pH 1) with hydrochloric acid 1N (35 ml), the aqueous layer was saturated with sodium chloride then was extracted with ether (3 x 100 ml). The combined organic layers were washed with brine (100 ml) dried over magnesium sulfate and evaporated to dryness.

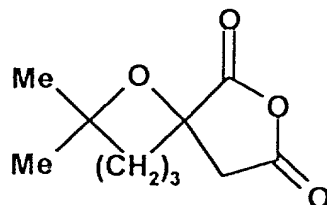
20

25 The resulting crude product (3.9 g) was purified by column chromatography (dichloromethane / methanol (99:1), silica (15–40  $\mu\text{m}$ ) 160 g) to provide a yellow oil (3.1 g, 67%). The crude product thus obtained showed identical characteristics to this obtained above.

30

## Example 7

### Preparation of cyclic anhydride of 2-carboxymethyl-2-hydroxy-6-methylhept-5-enoic acid



A mixture of cyclic diacid resulting from Example 4 (245 mg, 1.1 mmol) and acetic anhydride (4 ml) was stirred at reflux for 16 hours. After evaporation of reaction mixture *in vacuo*, the residue was treated with toluene and evaporated again in high vacuum to afford a viscous yellow oil (189 mg, 84%). The product thus obtained showed the following characteristics:

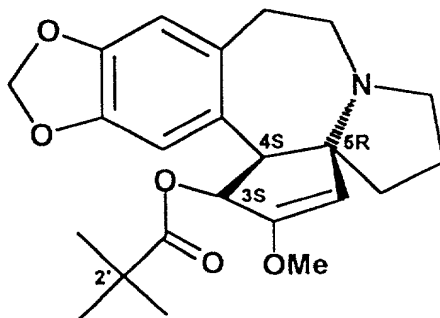
IR (ATR) ( $\text{cm}^{-1}$ ): 2976; 2951; 1732; 1188; 1170

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

3.02 (2H, s,  $\text{CH}_2\text{CO}_2$ ); 1.98 (2H, m,  $\text{CH}_2$ ); 1.8 - 1.5 (4H, m,  $\text{CH}_2$ ); 1.31 (3H, s,  $\text{CH}_3$ ); 1.22 (3H, s,  $\text{CH}_3$ ).

### Example 8

#### Preparation of (-)-cephalotaxyl pivalate:



#### 20 1°) Method via mixed anhydride

To a stirred mixture of pivalic acid (100 mg, 0.98 mmol) in anhydrous toluene (2 ml) was added at room temperature triethylamine (dried over potassium hydroxide) (138  $\mu\text{l}$ , 0.98 mmol) and 2,4,6-trichlorobenzoyl chloride (153  $\mu\text{l}$ , 0.98

mmol). After stirring at  $18 \pm 5^\circ\text{C}$  for 1.5 hours (with control of disappearing of starting acid in infra-red), 4-dimethylaminopyridine (139 mg, 1.14 mmol) was added the reaction mixture was allowed to react for 5 minutes and cephalotaxine (103 mg, 0.33 mmol) was added. After stirring at  $18 \pm 5^\circ\text{C}$  for 15 hours, the reaction mixture was filtered on paper and diluted with ether (5 ml). The resulting organic layer was successively washed with water (5 ml), with saturated sodium hydrogen carbonate solution (5 ml), with water again (5 ml) then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40  $\mu\text{m}$ )) to provide a solid (130 mg, 93%). The product thus obtained showed the following characteristics:

**$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):**

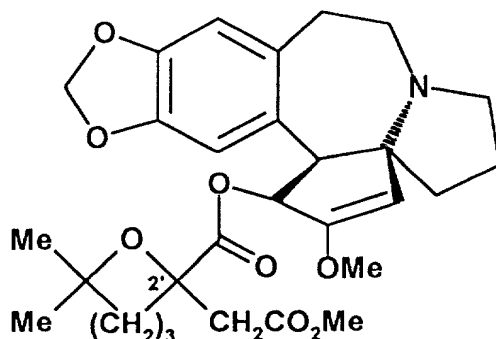
6.60 (1H, s, H-17\*); 6.58 (1H, s, H-14\*); 5.84 et 5.83 (2H, 2d,  $J_{\text{AB}} = 1.5$ ,  $\text{OCH}_2\text{O}$ ); 5.83 (1H, d, H-3); 5.02 (1H, s, H-1); 3.77 (1H, d,  $J_{4-3} = 9.6$ , H-4); 3.69 (3H, s,  $\text{OCH}_3$ ); 3.21 (1H, m,  $J_{\text{AB}} = 14.0$ ,  $J = 12.5$ , 7.8, H-11b); 3.09 (1H, m, H-8a); 2.94 (1H, td,  $J = 11.5$ , 7.1, H-10a); 2.57 (2H, m, H-8b + H-10b); 2.35 (1H, dd,  $J_{\text{AB}} = 14.5$ ,  $J = 6.9$ , H-11a); 2.03 (1H, td,  $J_{\text{AB}} = 12.1$ ,  $J = 9.7$ , H-6<sub>A</sub>); 1.89 (1H, m,  $J_{\text{AB}} = 12.1$ ,  $J = 7.9$ , 4.0, H-6<sub>B</sub>); 1.75 (2H, m,  $\text{CH}_2$ -7); 0.83 (9H, s,  $\text{C}(\text{CH}_3)_3$ ).

## **2°) Method using DCC**

To a stirred mixture of pivalic acid (50 mg, 0.49 mmol) in anhydrous toluene (2 ml) maintained in an inert atmosphere was added 1,3-dicyclohexylcarbodiimide (130 mg, 0.63 mmol). After stirring for 10 minutes at room temperature, cephalotaxine (50 mg, 0.16 mmol) and pyrrolidinopyridine (24 mg, 0.16 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 2 hours, then at  $50^\circ\text{C}$  for 15 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 9:1), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product (130 mg) was purified by column chromatography (dichloromethane / methanol (9:1), silica (15-40  $\mu\text{m}$ ) 3g) to provide a white solid (36 mg, 57%). The crude product thus obtained showed identical characteristics to this obtained above via mixed anhydride.

**Example 9**

Preparation of (-)-cephalotaxyl 2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydropyrane carboxylate or *anhydrohomoharringtonine* and methyl 2-cephalotaxyloxy-carbonylmethyl-6,6-dimethyl-2-tetrahydropyrane carboxylate, from cyclic anhydride resulting from Example 7:



To a stirred mixture of anhydride resulting from Example 7 (50 mg, 0.24 mmol) in anhydrous dichloromethane (0.5 ml) at room temperature were successively added pyridine (250  $\mu$ l, 3.1 mmol), pyrrolidinopyridine (10 mg, 0.07 mmol) and cephalotaxine (76.4 mg, 0.24 mmol). After stirring at  $18 \pm 5^\circ\text{C}$  for 48 hours, were successively added 1,3-dicyclohexylcarbodiimide (100 mg, 0.48 mmol), methanol (60  $\mu$ l, 1.5 mmol), pyrrolidinopyridine (10 mg, 0.07 mmol) and toluene (1 ml). After stirring at  $18 \pm 5^\circ\text{C}$  for 24 hours (with control of reaction in CCM), the reaction mixture was filtered and the filtrate was evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (99:1), silica (15–40  $\mu$ m)) to provide expected product (12 mg, two diastereomers) contaminated with regioisomer\* (two diastereomers) resulting from the opening of anhydride. The expected product thus obtained showed the following characteristics:

**$^1\text{H}$  NMR** 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

6.61 (1H, s, H-17\*); 6.57 (1H, s, H-14\*); 5.91 ( $J_{3-4} = 9.8$ ) and 5.84 (2H, 2d, H-3); 5.84 et 5.79 (2d,  $J_{AB} = 1.4$ ,  $\text{OCH}_2\text{O}$ ); 5.84 and 5.82 (2d,  $J_{AB} = 1.4$ ,  $\text{OCH}_2\text{O}$ ); 5.04 and 5.01 (1H, 2s, H-1); 3.79 and 3.78 (1H, 2d,  $J_{4-3} = 9.6$ , H-4); 3.70 and 3.65 (3H, 2s,  $\text{OCH}_3$ ); 3.59 (3H, s,  $\text{OCH}_3$ ); 3.15 (1H, m, H-11 $\beta$ ); 3.09 (1H, m, H-8 $\alpha$ ); 2.94 (1H, m, H-10 $\alpha$ ); 2.58 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.37 (1H, m, H-11 $\alpha$ ); 2.16



and 1.81 (2d,  $J_{AB} = 14.4$ ,  $\underline{\text{CH}_2\text{CO}_2}$ ); 2.13 and 1.66 (2d,  $J_{AB} = 14.3$ ,  $\underline{\text{CH}_2\text{CO}_2}$ ); 2.02 (1H, m, H-6<sub>A</sub>); 1.88 (1H, m, H-6<sub>B</sub>); 1.75 (2H, m,  $\text{CH}_2$ -7); 1.8 - 1.2 (6H, m, 3 x  $\text{CH}_2$ ); 1.11 and 1.02 (2s, 2 x  $\text{CH}_3$ ); 1.10 and 1.04 (2s, 2 x  $\text{CH}_3$ ).

- 5 \*The regioisomer above-mentioned was also obtained from the following conditions:

To a stirred mixture of hemiester resulting from Example 6 method C (100 mg, 0.43 mmol) in anhydrous toluene (1 ml) maintained in an inert atmosphere at room temperature was added 1,3-dicyclohexylcarbodiimide (120 mg, 0.58 mmol). After stirring for 5 minutes, cephalotaxine (45 mg, 0.15 mmol) and pyrrolidinopyridine (21 mg, 0.14 mmol) were added. After stirring at 35°C for 45 minutes, then at 8°C for 15 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 9:1), the reaction mixture was filtered, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40  $\mu\text{m}$ ) 4g) to provide expected product (23 mg, 30%, two diastereomers). The product thus obtained showed the following characteristics:

- 20 <sup>1</sup>H NMR 400 MHz ( $\text{CDCl}_3$ ) (  $\delta$  ppm, J Hz):  
 6.61 and 6.58 (1H, 2s, H-17\*); 6.57 and 6.53 (1H, 2s, H-14\*); 5.89 and 5.86 (2d,  $J_{AB} = 1.5$ ,  $\text{OCH}_2\text{O}$ ); 5.87 and 5.85 (2d,  $J_{AB} = 1.5$ ,  $\text{OCH}_2\text{O}$ ); 5.76 (1H, d,  $J_{3-4} = 9.4$ , H-3); 5.02 (1H, 2s, H-1); 3.73 and 3.72 (1H, 2d,  $J_{4-3} = 9.4$ , H-4); 3.70 and 3.68 (3H, 2s,  $\text{OCH}_3$ ); 3.69 and 3.65 (3H, 2s,  $\text{OCH}_3$ ); 3.15 (1H, m, H-11 $\alpha$ ); 3.07 (1H, m, H-8 $\alpha$ ); 2.90 (1H, m, H-10 $\alpha$ ); 2.74 and 1.95 (2d,  $J_{AB} = 15.3$ ,  $\underline{\text{CH}_2\text{CO}_2}$ ); 2.56 (2H, m, H-8 $\beta$ + H-10 $\beta$ ); 2.33 (1H, m, H-11 $\alpha$ ); 2.28 and 2.23 (2d,  $J_{AB} = 15.4$ ,  $\underline{\text{CH}_2\text{CO}_2}$ ); 2.16 (m, H-3'<sub>eq</sub>); 1.97 (1H, m, H-6<sub>A</sub>); 1.9 - 1.1 (5H, m,  $\text{CH}_2$ ); 1.86 (1H, m, H-6<sub>B</sub>); 1.73 (2H, m,  $\text{CH}_2$ -7); 1.14 (3H, s,  $\text{CH}_3$ ); 1.03 (3H, s,  $\text{CH}_3$ ).

30

### Example 10

- Preparation of (-)-cephalotaxyl 2-methoxycarbonylmethyl-6,6-dimethyl-2 tetrahydropyrane carboxylate or anhydrohomoharringtonine, from tetrahydropyranecarboxylic acid resulting from Example 6:
- 35

## FORMULA OF EXAMPLE 9

**1°) Method via mixed anhydride**

To a stirred mixture of hemiester resulting from Example 6 (50 mg, 0.22 mmol) in anhydrous toluene (1 ml) at room temperature was added triethylamine (dried over potassium hydroxide) (29.4  $\mu$ l, 0.22 mmol) and 2,4,6-trichlorobenzoyl chloride (32.7  $\mu$ l, 0.22 mmol). After stirring at 25°C for 20 hours (with control of disappearing of starting acid in infra-red), 4-dimethylaminopyridine (29 mg, 0.24 mmol) was added, the reaction mixture was allowed to react for 5 minutes and cephalotaxine (16.5 mg, 0.05 mmol) was added. After stirring at 25°C for 24 hours, the reaction mixture was filtered on paper and diluted with ether (5 ml). The resulting organic layer was successively washed with water (5 ml), with saturated sodium hydrogen carbonate solution (5 ml), with water again (5 ml), then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40  $\mu$ m)) to provide expected product (16 mg, 56%, two diastereomers). The product thus obtained showed identical characteristics to this obtained in Example 9.

**2°) Method using DCC**

To a stirred mixture of hemiester resulting from Example 6 (100 mg, 0.43 mmol) in anhydrous toluene (1 ml) maintained in an inert atmosphere at room temperature was added 1,3-dicyclohexylcarbodiimide (180 mg, 0.87 mmol). After stirring for 10 minutes, cephalotaxine (165 mg, 0.52 mmol) and pyrrolidinopyridine (77 mg, 0.52 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 18 hours, was added ether, the reaction mixture was filtered on ground-glass filter, and the cake was washed with ether. The resulting organic layer was successively washed with 10% sodium hydrogen carbonate solution, with water, then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40  $\mu$ m) 9 g) to provide a solid (110 mg, 48%). The product thus obtained showed identical characteristics to this obtained in Example 9.

**Example 11**

5 **Preparation of (-)-cephalotaxyl (2'RS)-2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydropyrane carboxylate or anhydrohomoharringtonine, from ethylenic acid resulting from Example 6:**

**FORMULA OF EXAMPLE 9**10 **1°) Method A: via mixed anhydride, coupling with cyclisation**

To a stirred mixture of ethylenic ester resulting from Example 5 (50 mg, 0.22 mmol) in anhydrous toluene (1 ml) at room temperature was added triethylamine (dried over potassium hydroxide) (29  $\mu$ l, 0.22 mmol) and 2,4,6-trichlorobenzoyl chloride (34  $\mu$ l, 0.22 mmol). After stirring for 30 minutes (with  
15 control of disappearing of starting acid in infra-red), 4-dimethylaminopyridine (30 mg, 0.25 mmol) was added, the reaction mixture was allowed to react for 5 minutes and cephalotaxine (31 mg, 0.1 mmol) was added. After stirring at  $18 \pm 5^\circ\text{C}$  for 65 hours, the reaction mixture was filtered on paper and diluted with ether (5 ml). The resulting organic layer was successively washed with water  
20 (5 ml), with saturated sodium hydrogen carbonate solution (5 ml), with water again (5 ml), then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40  $\mu$ m)) to provide expected product (46 mg, 96%, two diastereomers 40/60). The product thus obtained  
25 showed identical characteristics to this obtained in Example 9.

**2°) Method B: using DCC, coupling with cyclisation**

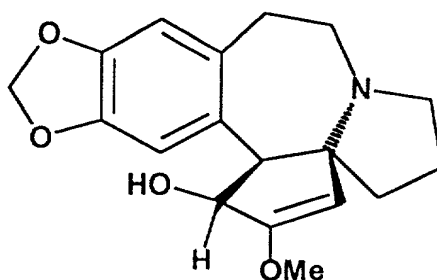
To a stirred mixture of ethylenic acid resulting from Example 5 (50 mg, 0.22  
30 mmol) in anhydrous toluene (2 ml) maintained in an inert atmosphere at room temperature was added 1,3-dicyclohexylcarbodiimide (270 mg, 1.31 mmol). After stirring for 5 minutes, cephalotaxine (70 mg, 0.22 mmol) and pyrrolidinopyridine (32 mg, 0.22 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 65 hours (with control of reaction in CCM, eluant dichloromethane /  
35 methanol; 9:1), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5ml) and the filtrate was evaporated *in vacuo*. The

resulting crude product was purified by column chromatography (dichloromethane / methanol (98:2), silica (15–40  $\mu\text{m}$ ) 9 g) to provide a solid (40 mg, 35%). The product thus obtained showed identical characteristics to this obtained in Example 9.

5

### Example 12

#### Preparation of purified (-) cephalotaxine from total alkaloidic extract of 10 *Cephalotaxus sp*



##### **1°) preparation of total alkaloids extract:**

In a 30 liters-tank, crushed leaves (fresh or dry) of *Cephalotaxus sp* (10 kg) were mixed with methanol (20l) and steed during 65 hours, then percolated (50l). Solution was filtered and concentrated under vacuum to a volume of 5l.

15 Concentrated solution was acidified with a 6% aqueous solution of tartaric acid. Then hydro-alcoholic solution was washed by dichloromethane (5  $\times$  5l) for removing fatty materials and pigments. Aqueous solution was basified with aqueous ammonia (2.5%) until pH 9, then extracted with dichloromethane (5  $\times$  5 l). After concentration under reduced pressure, crude alkaloids extract was  
20 recovered as a white crystalline solid (24.5 g). Cephalotaxine contain was 71% (HPLC).

##### **2°) Isolation and chromatographic purification of (-)-cephalotaxine from crude alkaloids extract:**

25 Above crude extract was dissolved in mobile phase (triethylamine (1.55 / 1000) in deionised water and orthophosphoric acid to adjust pH to 3. The solution was filtered then injected on a preparative high-performance liquid chromatograph equipped with axial compression and high pressure pump (stationary phase : n-octadecylsilane, 15  $\mu\text{m}$ , porosity 100, 1kg). Elution was performed at a flow

rate of 0.2 l/min. Fractions contain was monitored by U.V. detector and TLC. Retained fraction were finally checked by HPLC then combined, alkalinised with 2.5% aqueous ammonia and extracted with dichloromethane (4 x 400 ml), After concentration under reduced pressure a resin was obtained which on trituration with methanol gave (-)-cephalotaxine (18g) as a white crystalline solid ( HPLC purity = 99.8%). The product thus obtained showed the following characteristics :

$[\alpha]_D^{20}$ : -174,1 (c = 0,20; CHCl<sub>3</sub>)

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

6.68 (1H, s, H-17\*); 6.65 (1H, s, H-14\*); 5.91 and 5.90 (2H, 2d, J<sub>AB</sub> = 1.5, OCH<sub>2</sub>O); 4.93 (1H, s, H-1); 4.77 (1H, dd, J<sub>3-4</sub> = 9.4, J<sub>3-OH</sub> = 3.4, H-3); 3.73 (3H, s, OCH<sub>3</sub>); 3.68 (1H, d, J<sub>4-3</sub> = 9.4, H-4); 3.35 (1H, m, J<sub>AB</sub> = 14.3, J = 12.2 and 7.9, H-11β); 3.08 (1H, m, J = 9.1 and 4.9, H-8α); 2.92 (1H, td, J = 11.6 and 7.1, H-10α); 2.59 (2H, m, H-8α + H-10α); 2.35 (1H, dd, J<sub>AB</sub> = 14.4, J = 6.9, H-11α); 2.02 (1H, td, J<sub>AB</sub> = 12.1, J = 9.7, H-6<sub>A</sub>); 1.87 (1H, m, J<sub>AB</sub> = 12.1, J = 7.9 and 4.4, H-6<sub>B</sub>); 1.74 (2H, m, CH<sub>2</sub>-7); 1.62 (1H, d, J<sub>3-OH</sub> = 3.5, 3-OH).

### Example 13

#### Preparation of lithium alcoolate of (-) cephalotaxine (trapped like 3-O-acetyl derivative)

##### **1°) Butyllithium Method**

A commercial solution of butyllithium in hexane (0.44 ml, 1.6 M in hexane, 0.70 mmol) was added to a stirred mixture of (-)-cephalotaxine (200 mg, 0.63 mmol) in anhydrous tetrahydrofurane (6.8 ml). The reaction mixture was maintained at -60°C for 20 minutes, then at -48°C for 30 minutes, acetic anhydride (90 μl, .095 mmol) was added over a period of 8 minutes and the stirring was maintained at -48°C for 20 minutes then at 0°C for 1 hour. The mixture was quenched with saturated ammonium chloride solution (5 ml) then extracted with ethyl acetate (3 x 8 ml). The combined organic layers were washed with brine (15 ml) dried over magnesium sulfate and evaporated to dryness. The resulting crude product was purified by column chromatography (dichloromethane /

methanol (98:2), silica (15–40  $\mu\text{m}$ ) 6 g) to provide a white solid (60 mg, 26%). The product thus obtained showed the following characteristics:

**$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):**

5 6.60 (1H, s, H-17\*); 6.57 (1H, s, H-14\*); 5.89 and 5.86 (2H, 2d,  $J_{\text{AB}} = 1.4$ ,  $\text{OCH}_2\text{O}$ ); 5.80 (1H, d,  $J_{3-4} = 9.3$ , H-3); 5.05 (1H, s, H-1); 3.77 (1H, d,  $J_{4-3} = 9.4$ , H-4); 3.72 (3H, s,  $\text{OCH}_3$ ); 3.23 (1H, m,  $J_{\text{AB}} = 14.3$ ,  $J = 12.3$  and  $7.9$ , H-11 $\beta$ ); 3.08 (1H, m, H-8 $\alpha$ ); 2.92 (1H, td,  $J = 11.5$  and  $7.1$ , H-10 $\alpha$ ); 2.57 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.36 (1H, dd,  $J_{\text{AB}} = 14.4$ ,  $J = 7.0$ , H-11 $\alpha$ ); 2.02 (1H, td,  $J_{\text{AB}} = 12.1$ ,  $J = 9.7$ , H-6 $\text{A}$ ); 1.88 (1H, m,  $J_{\text{AB}} = 12.1$ ,  $J = 8.0$  and  $4.0$ , H-6 $\text{B}$ ); 1.74 (2H, m,  $\text{CH}_2$ -7); 1.57 (3H, s,  $\text{OAc}$ ).

### **2°) Lithium bis-(trimethylsilyl)amide (LHDS) method**

15 A commercial solution of lithium bis-(trimethylsilyl)amide 1M in tetrahydrofuran (0.95 ml, 0.95 mmol) was added to a stirred solution of (-)-cephalotaxine (200 mg, 0.63 mmol) in anhydrous tetrahydrofuran at  $-40^\circ\text{C}$ . After stirring for 5 minutes, acetic anhydride (90  $\mu\text{l}$ , 0.95 mmol) was added, and the reaction mixture was treated like method above-mentioned in 1°. The product thus obtained showed identical characteristics to this obtained above in butyllithium method.

### **3°) Lithium diisopropylamide (LDA) method**

25 A commercial solution of lithium diisopropylamide 2M in tetrahydrofuran (0.35 ml, 0.70 mmol) was added to a stirred solution of (-)-cephalotaxine (200 mg, 0.63 mmol) in anhydrous tetrahydrofuran (6.8 ml) at  $-60^\circ\text{C}$  over a period of 20 minutes. After stirring at  $-60^\circ\text{C}$  for 20 minutes, then at  $-48^\circ\text{C}$  for 30 minutes, acetic anhydride (90  $\mu\text{l}$ , 0.95 mmol) was added. The solution was stirred at  $-48^\circ\text{C}$  for 20 minutes, then at  $0^\circ\text{C}$  for 1 hour and the reaction mixture was treated like method above-mentioned in 1°. The product thus obtained showed identical characteristics to this obtained above in butyllithium method.

### **4°) Sodium hydride Method**

35 To a stirred mixture of sodium hydride (1.5 g) in freshly distilled dimethylformamide (3 ml) were added at  $-60^\circ\text{C}$  a solution of cephalotaxine (200 mg, 0.63 mmol) in dimethylformamide (3 ml) and acetic anhydride (90  $\mu\text{l}$ , 0.95 mmol). After stirring at ambient temperature for 24 hours, the reaction mixture was treated at  $0^\circ\text{C}$  with water (3 ml) and extracted with ether ( $3 \times 5$  ml). The combined organic layers were dried over magnesium sulfate and

evaporated on vacuum. The product thus obtained showed identical characteristics to this obtained above in butyllithium method.

5

#### Example 14

Preparation of (-)-cephalotaxyl 2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydropyrane carboxylate or anhydrohomoharringtonine, via lithium  
10 alcoolate of cephalotaxine:

#### FORMULA OF EXAMPLE 9

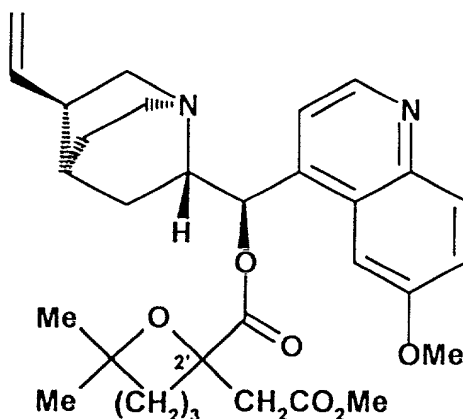
To a stirred solution of lithium alcoolate of (-)-cephalotaxine (158 mg, 0.5 mmol)  
15 in anhydrous tetrahydrofuran prepared according to Example 13 was added  
mixed anhydride resulting from Example 10 (0.75 mmol) at -50°C over a period  
of 10 minutes. After stirring at -50°C for 30 minutes, then at 0°C for 2 hours,  
the reaction mixture was quenched with saturated ammonium chloride solution  
20 (5 ml) and extracted with ethyl acetate (3 x 10 ml). The combined organic  
layers were washed with brine (15 ml) dried over magnesium sulfate and  
evaporated *in vacuo*. The resulting crude product was purified by column  
chromatography (dichloromethane / methanol (98:2), silica (15-40 µm) 7 g) to  
provide a white solid (48 mg). The product thus obtained showed identical  
characteristics to this obtained in Example 9.

25

#### Example 15

Preparation of diastereomeric mixture of (-)-quinidyl 2-methoxycarbonyl-  
30 methyl-6,6-dimethyl-2-tetrahydropyrane carboxylates, from  
tetrahydropyranecarboxylic acid resulting from Example 6:

09270006-034699



### 1°) Method A: via mixed anhydride

To a stirred mixture of acid resulting from Example 6 (458 mg, 1.99 mmol) in anhydrous toluene (8 ml) at room temperature was added triethylamine (dried over potassium hydroxide) (270  $\mu$ l, 1.92 mmol) and 2,4,6-trichlorobenzoyl chloride (300  $\mu$ l, 1.91 mmol). After stirring for 3 hours (with control of disappearing of starting acid in infra-red), 4-dimethylaminopyridine (352 mg, 2.88 mmol) was added, the reaction mixture was allowed to react for 5 minutes and quinine (936 mg, 2.88 mmol) was added. After stirring at  $18 \pm 5^\circ\text{C}$  for 65 hours, the reaction mixture was filtered on paper and diluted with ether (15 ml).

The resulting organic layer was successively washed with water (15 ml), with saturated sodium hydrogen carbonate solution (15 ml), with water again (15 ml) then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (99:1), silica (15–40  $\mu$ m) 32g) to provide expected product (930 mg, 84%, two diastereomers 50/50). The product thus obtained showed the following characteristics:

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (  $\delta$  ppm, J Hz):

8.73 (1H, m, H-2<sub>qn</sub>), 8.0 and 7.98 (1H, 2d, J = 9.2, H-8<sub>qn</sub>); 7.63 and 7.50 (1H, 2br.s); 7.45 (br.s) and 7.39 (d, J = 4.5) (1H, H-3<sub>qn</sub>); 7.36 (1H, dd, J = 9.1 and 2.6, H-7<sub>qn</sub>); 6.50 (1H, br.s); 5.89 (1H, m, =CH<sub>qn</sub>); 5.03 (2H, m, =CH<sub>2qn</sub>); 3.99 and 3.97 (3H, 2s, OCH<sub>3</sub>); 3.54 and 3.33 (3H, 2br.s, OCH<sub>3</sub>); 3.2 - 1.0 (m, 7 $\times$ CH<sub>2</sub> + 3CH); 2.92 and 2.67 (2d, J<sub>AB</sub> = 14.9, CH<sub>2</sub>CO<sub>2</sub>); 2.87 (d, J<sub>AB</sub> = 14.8, CH<sub>2</sub>CO<sub>2</sub>); 1.17 and 0.99 (2s, 2  $\times$  CH<sub>3</sub>); 1.03 and 0.42 (2br.s, 2  $\times$  CH<sub>3</sub>).

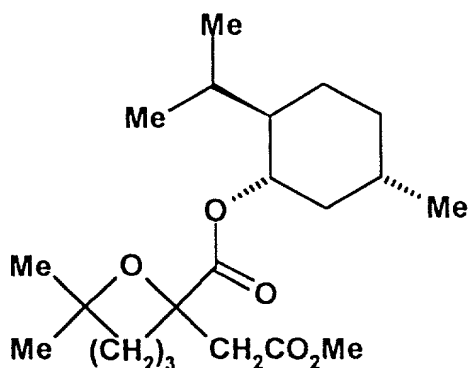


**2°) Method B: DCC**

To a stirred mixture of tetrahydrocarboxylic acid resulting from Example 6 (200 mg, 0.87 mmol) in anhydrous toluene (4 ml) maintained in an inert atmosphere at room temperature was added 1,3-dicyclohexylcarbodiimide (239 mg, 1.16 mmol). After stirring for 5 minutes, quinine (94 mg, 0.29 mmol) and pyrrolidinopyridine (43 mg, 0.29 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 65 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 9:1), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (9:1), silica (15–40  $\mu\text{m}$ )) to provide the expected product (96 mg, 60%, two diastereomers 50/50). The product thus obtained showed identical characteristics to this obtained above.

**Example 16**

**Preparation of (-)-menthyl 2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydro-pyrane carboxylate, tetrahydropyrane-carboxylic acid resulting from Example 6:**



To a stirred mixture of acid resulting from Example 6 (50 mg, 0.22 mmol) in anhydrous toluene (1 ml) maintained in an inert atmosphere at room temperature was added 1,3-dicyclohexylcarbodiimide (90 mg, 0.44 mmol). After stirring for 5 minutes, (-)-menthol (68 mg, 0.44 mmol) and pyrrolidinopyridine (64 mg, 0.44 mmol) were added. After stirring at  $30^\circ\text{C}$  for 1 hour, then at  $8^\circ\text{C}$  for 15 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 9:1), the reaction mixture was filtered on ground-glass filter, the cake

was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product was purified by column chromatography (cyclohexane / ethyl acetate (95:5 then 90:10), silica (15-40  $\mu\text{m}$ ) 4 g) to provide the expected product (40 mg, 50%, two diastereomers 60/40). The product thus obtained

5 showed the following characteristics:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

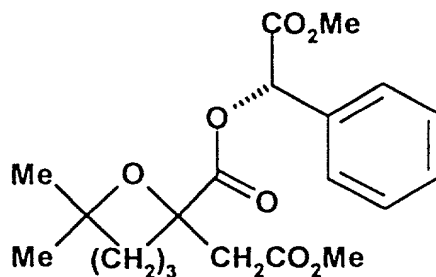
4.68 (1H, m,  $\text{H-1}_{\text{men}}$ ); 3.64 (3H, s,  $\text{OCH}_3$ ); 2.84 and 2.64 (2d,  $J_{\text{AB}} = 14.6$ ,  $\text{CH}_2\text{CO}_2$ ); 2.83 and 2.63 (2d,  $J_{\text{AB}} = 14.3$ ,  $\text{CH}_2\text{CO}_2$ ); 2.29 (1H, m,  $\text{H-3}_{\text{eq}}$ ); 2.1 - 0.8 (m, CH and  $\text{CH}_2$ ); 1.21 (3H, 2s,  $\text{CH}_3$ ); 1.17 and 1.16 (3H, 2s,  $\text{CH}_3$ ); 0.9 and 0.88 (6H, 2d,  $J = 6.4$ ,  $2 \times \text{CH}_3_{\text{men}}$ ); 0.74 and 0.72 (3H, 2d,  $J = 6.8$ ,  $\text{CH}_3_{\text{men}}$ ).

10

### Example 17

15

Preparation of (-)-methyl mandelate 2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydropyran carboxylate from tetrahydropyranecarboxylic acid resulting from Example 6:



20

To a stirred mixture of tetrahydropyranecarboxylic acid resulting from Example 6 (226 mg, 0.98 mmol) in anhydrous toluene (4 ml) maintained in an inert atmosphere at room temperature was added 1,3-dicyclohexylcarbodiimide (261 mg, 1.2 mmol). After stirring for 5 minutes, menthyl mandelate (53 mg, 0.32 mmol) and pyrrolidinopyridine (47 mg, 0.32 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 12 hours, the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product was purified by column chromatography

25

(dichloromethane / methanol (9:1), silica (15–40  $\mu\text{m}$ )) to provide a colorless oil (64 mg, 17%, two diastereomers). The product thus obtained showed the following characteristics:

5  $^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

7.47 (2H, m, Ph); 7.38 (3H, m, Ph); 5.96 (1H, s, CH); 3.73 and 3.72 (3H, 2s,  $\text{OCH}_3$ ); 3.54 (3H, 2s,  $\text{OCH}_3$ ); 2.88 and 2.72 (2d,  $J_{\text{AB}} = 14.4$ ,  $\text{CH}_2\text{CO}_2$ ); 2.85 and 2.65 (2d,  $J_{\text{AB}} = 14.2$ ,  $\text{CH}_2\text{CO}_2$ ); 2.35 (1H, m, H-3 $_{\text{eq}}$ ); 2.0 - 1.15 (5H, m,  $\text{CH}_2$ ); 1.23 and 1.22 (3H, 2s,  $\text{CH}_3$ ); 1.19 and 1.07 (3H, 2s,  $\text{CH}_3$ ).

10

### Example 18

#### Separation of (-)-quinyll (2'R)-anhydrohomoharringtonate and (-)-quinyll (2'S)-anhydrohomoharringtonate from diastereomeric mixture resulting from Example 15:

15

Diastereomeric mixture of (-)-quinyll (2'R)-anhydrohomoharringtonate and (-)-quinyll (2'S)-anhydrohomoharringtonate (5g) was submit to preparative HPLC.

20

Above mixture was dissolved in buffer (triethylamine (1.55 / 1000) in deionised water and orthophosphoric acid to adjust pH to 3. The solution was filtered then injected on a preparative high-performance liquid chromatograph equipped with axial compression and high pressure pump (stationary phase: n-octadecylsilane, 15  $\mu\text{m}$ , porosity 100, 1kg; mobile phase: buffer / acetonitrile

25

70 / 30). Elution was performed at a flow rate of 0.2 l/min. Fractions contain was monitored by U.V. detector and TLC. Retained fraction were finally checked by HPLC then combined, alkalinised with 2.5% aqueous ammonia and extracted with dichloromethane (4 x 400 ml), After concentration under reduced pressure the two separated isomers were obtained as white crystalline solids corresponding to (-)-quinyll (2'R)-anhydrohomoharringtonate (2g) and (-)-quinyll (2'S)-anhydrohomoharringtonate (2.2g). The products thus obtained showed the following characteristics :

30

#### 1°) Diastereomer 2'R

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

10

15

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

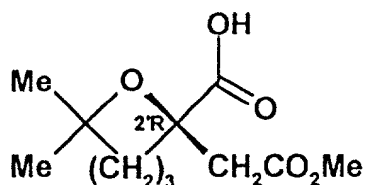
8.74 (1H, d, J = 4.4, H-2<sub>qn</sub>), 7.99 (1H, d, J = 9.2, H-8<sub>qn</sub>); 7.65 (1H, br.s, H-3<sub>qn</sub>); 7.44 (1H, br.s, H-5<sub>qn</sub>); 7.36 (1H, dd, J = 9.2 and 2.7, H-7<sub>qn</sub>); 6.55 (1H, br.s); 5.89 (1H, m, =CH<sub>qn</sub>); 5.05 (2H, m, =CH<sub>2qn</sub>); 3.99 (3H, s, OCH<sub>3</sub>); 3.54 (3H, s, OCH<sub>3</sub>);

3.1 - 1.0 (m, 7 x CH<sub>2</sub> + 3CH); 2.91 and 2.67 (2H, 2d, J<sub>AB</sub> = 15.0, CH<sub>2</sub>CO<sub>2</sub>); 1.03 (3H, br.s, CH<sub>3</sub>); 0.44 (3H, br.s, CH<sub>3</sub>).

5

### Example 19

**Preparation of (2R)-anhydrohomoharringtonic acid from (-)-quinyl (2'R)-anhydrohomoharringtonate resulting from Example 18:**



10

#### 1°) Obtaining via hydrogenolysis

To a stirred solution of quinyl (2'R)-anhydroharringtonate (100 mg, 0.19 mmol) in ethyl acetate (11 ml) was added 10% palladium on charcoal (40 mg). The resulting mixture was stirred at room temperature under hydrogen pressure (50 p.s.i.) for 20 hours, and after CCM control the reaction mixture was filtered and the resulting organic layer was treated with saturated sodium hydrogen carbonate solution. The aqueous layer was washed with ethyl acetate, and after acidification with hydrochloric acid 1N was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate and evaporated to dryness to afford a yellow solid (20 mg, 50%). The product thus obtained showed the following characteristics:

15

$[\alpha]_D^{20}$ : -23 (c = 0,38; CHCl<sub>3</sub>).

20

IR (film NaCl) (cm<sup>-1</sup>): 2974; 2951; 1740; 1718; 1437.

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz): <sup>1</sup>H NMR spectra of the product thus obtained was identical to this described in Example 6-1.

25

#### 2°) Obtaining via total saponification then selective methylation

A mixture of potassium hydroxide (396 mg, 7.1 mmol) in water (8 ml) was added

30

to a stirred solution of quinyll (2'R)-anhydroharringtonate (396 g, 0.72 mmol) in ethanol (15 ml) and the resulting mixture was stirred at reflux for 24 hours. After cooling at room temperature and removal of ethanol *in vacuo*, the residue was treated with water (10 ml) and the resulting aqueous layer was extracted with ether (4 × 15 ml). After acidification (pH 1) with hydrochloric acid 2N and saturation with sodium chloride, the aqueous layer was extracted with ethyl acetate (3 × 15 ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a yellow solid (110 mg, 72%). The intermediate diacid thus obtained showed the following characteristics:

$[\alpha]_D^{20}$ : -14 (c = 0,54; CHCl<sub>3</sub>)

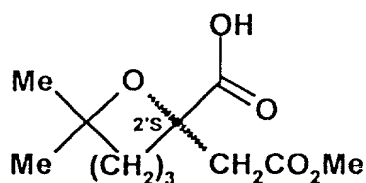
IR (film NaCl) (cm<sup>-1</sup>): 2975; 2941; 1716; 1217.

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz): <sup>1</sup>H NMR spectra of the product thus obtained was identical to this described in Example 4.

A mixture of (2R)-cyclic diacid above-mentioned (110 mg, 0.5 mmol) and a commercial solution of boron trifluoride-methanol complex in methanol (1.1 ml, BF<sub>3</sub> 12% w/w) was stirred at 18 ± 5°C for 15 hours. After careful addition of the reaction mixture at saturated sodium hydrogen carbonate solution (20 ml), the resulting aqueous layer was washed with ether (3 × 15 ml), acidified (pH 1) with hydrochloric acid 2N, and extracted with ether (3 × 15 ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a viscous yellow oil (69 mg, 59%). The product thus obtained showed identical characteristics to this obtained from method 1° above.

### Example 20

**Preparation of (2S)-anhydrohomoharringtonic acid from (-)-quinyll (2'R)-anhydrohomoharringtonate resulting from Example 18:**



### 1°) Obtaining via hydrogenolysis

To a stirred solution of quinyll (2'S)-anhydroharringtonate (100 mg, 0.19 mmol) in ethyl acetate (11 ml) was added 10% palladium on charcoal (40 mg). The resulting mixture was stirred at room temperature under hydrogen pressure (50 p.s.i.) and after CCM control, the reaction mixture was filtered and the resulting organic layer was treated with saturated sodium hydrogen carbonate solution. The aqueous layer was washed with ethyl acetate and after acidification with hydrochloric acid 1N was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over magnesium sulfate and evaporated to dryness to afford a yellow solid (23 mg, 53%). The product thus obtained showed the following characteristics:

$[\alpha]_D^{20}$ : +30 ( $c = 0,36$ ;  $\text{CHCl}_3$ )

IR (film NaCl) ( $\text{cm}^{-1}$ ): 2975; 2951; 1740; 1718; 1439.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):  $^1\text{H}$  NMR spectra of the product thus obtained was identical to this described in Example 6-1.

### 2°) Obtaining via total saponification then selective methylation

A mixture of potassium hydroxide (430 mg, 7.7 mmol) in water (9 ml) was added to a stirred solution of quinyll (2'S)-anhydroharringtonate (447 g, 0.81 mmol) in ethanol (16 ml) and the resulting mixture was stirred at reflux for 24 hours. After cooling at room temperature and removal of ethanol *in vacuo*, the residue was treated with water (10 ml) and the resulting aqueous layer was extracted with ether ( $4 \times 15$  ml). After acidification (pH 1) with hydrochloric acid 2N and saturation with sodium chloride, the aqueous layer was extracted with ethyl acetate ( $3 \times 15$  ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a yellow solid (140 mg, 80%). The cyclic diacid thus obtained showed the following characteristics:

$[\alpha]_D^{20}$ : +8 ( $c = 0,19$ ;  $\text{CHCl}_3$ )

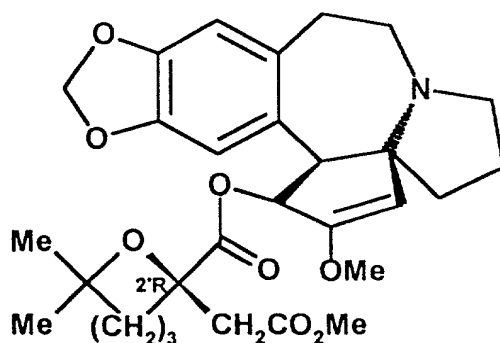
IR (film NaCl) ( $\text{cm}^{-1}$ ): 2975; 2945; 1717.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):  $^1\text{H}$  NMR spectra of the product thus obtained was identical to this described in Example 4.

A mixture of (2S)-cyclic diacid above-mentioned (136 mg, 0.62 mmol) and a commercial solution of boron trifluoride-methanol complex in methanol (1.3 ml,  $\text{BF}_3$  12% w/w) was stirred at  $18 \pm 5^\circ\text{C}$  for 15 hours. After careful addition of the reaction mixture at saturated sodium hydrogen carbonate solution (20 ml), the resulting aqueous layer was washed with ether ( $3 \times 15$  ml), acidified (pH 1) with hydrochloric acid 2N and extracted with ether ( $3 \times 15$  ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a viscous yellow oil (81 mg, 63%). The product thus obtained showed identical characteristics to this obtained from method 1° above.

### Example 21

Preparation of anhydrohomoharringtonine, via esterification of cephalotaxine with (2R)-(+)-anhydrohomoharringtonic acid



To a stirred mixture of hemiester (R) resulting from Example 19 (65 mg, 0.28 mmol) in anhydrous toluene (1 ml) at room temperature was added triethylamine (dried over potassium hydroxide) ( $38 \mu\text{l}$ , 0.28 mmol) and 2,4,6-trichlorobenzoyl chloride ( $43 \mu\text{l}$ , 0.28 mmol). After stirring at  $30^\circ\text{C}$  for 1.5 hours (with control of disappearing of starting acid in infra-red), 4-



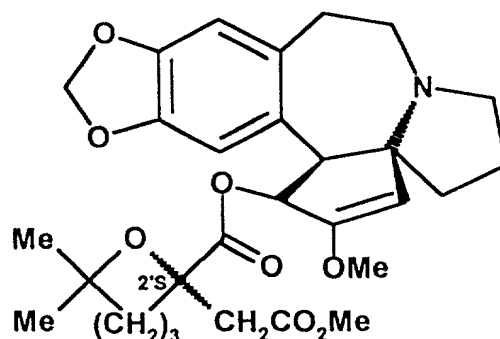
dimethylaminopyridine (50 mg, 0.41 mmol) was added, the reaction mixture was allowed to react for 5 minutes and cephalotaxine (129 mg, 0.41 mmol) was added. After stirring at 30°C for 18 hours, the reaction mixture was filtered on paper and diluted with ether (5 ml). The resulting organic layer was successively washed with water (5 ml), with saturated sodium hydrogen carbonate solution (5 ml), with water again (5 ml) then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (99:1), silica (15-40  $\mu$ m)) to provide expected product (65 mg, 43%). The product thus obtained showed following characteristics:

$^1\text{H NMR}$  400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

6.61 (1H, s, H-17\*); 6.58 (1H, s, H-14\*); 5.92 (1H, d,  $J_{3,4} = 9.6$ , H-3); 5.87 and 5.79 (2H, 2s,  $\text{OCH}_2\text{O}$ ); 5.04 (1H, br.s, H-1); 3.80 (1H, d,  $J_{4,3} = 9.2$ , H-4); 3.70 (3H, s,  $\text{OCH}_3$ ); 3.59 (3H, s,  $\text{OCH}_3$ ); 3.12 (2H, m, H-11 $\beta$  + H-8 $\alpha$ ); 2.95 (1H, m, H-10 $\alpha$ ); 2.60 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.38 (1H, m, H-11 $\alpha$ ); 2.13 and 1.66 (2H, 2d,  $J_{\text{AB}} = 14.3$ ,  $\text{CH}_2\text{CO}_2$ ); 2.02 (1H, m, H-6 $\text{A}$ ); 1.90 (1H, m, H-6 $\text{B}$ ); 1.76 (2H, m,  $\text{CH}_2$ -7); 1.8 - 1.2 (6H, m, 3 x  $\text{CH}_2$ ); 1.10 (3H, s,  $\text{CH}_3$ ); 1.04 (3H, s,  $\text{CH}_3$ ).

### Example 22

#### Preparation of anhydroepihomoharringtonine, via esterification of cephalotaxine with (2S)-(+)-anhydrohomoharringtonic acid



To a stirred mixture of hemiester (S) resulting from Example 20 (87 mg, 0.38 mmol) in anhydrous toluene (1.7 ml) at room temperature was added triethylamine (dried over potassium hydroxide) (52  $\mu$ l, 0.38 mmol) and 2,4,6-

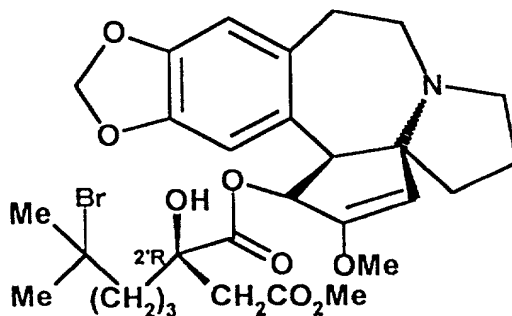
trichlorobenzoyl chloride (57  $\mu$ l, 0.38 mmol). After stirring at 30°C for 1.5 hours (with control of disappearing of starting acid in infra-red), 4-dimethylaminopyridine (70 mg, 0.57 mmol) was added, the reaction mixture was allowed to react for 5 minutes and cephalotaxine (180 mg, 0.57 mmol) was added. After stirring at 30°C for 18 hours, the reaction mixture was filtered on paper and diluted with ether (5 ml). The resulting organic layer was successively washed with water (5 ml), with saturated sodium hydrogen carbonate solution (5 ml), with water again (5 ml), then was dried over magnesium sulfate and evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane / methanol (99:1), silica (15-40  $\mu$ m)) to provide expected product (101 mg, 50%). The product thus obtained showed following characteristics:

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) ( $\delta$  ppm, J Hz):

6.61 (1H, s, H-17\*); 6.57 (1H, s, H-14\*); 5.84 (3H, m, H-3) + OCH<sub>2</sub>O); 5.04 (1H, s, H-1); 3.78 (1H, d, J<sub>4-3</sub> = 9.7, H-4); 3.65 (3H, s, OCH<sub>3</sub>); 3.59 (3H, s, OCH<sub>3</sub>); 3.23 (1H, m, H-11 $\beta$ ); 3.09 (1H, m, H-8 $\alpha$ ); 2.93 (1H, m, H-10 $\alpha$ ); 2.58 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.39 (1H, dd, J<sub>AB</sub> = 14.4, J = 7.0, H-11 $\alpha$ ); 2.16 and 1.83 (2H, 2d, J<sub>AB</sub> = 14.5, CH<sub>2</sub>CO<sub>2</sub>); 2.06 (1H, m, H-6 $\alpha$ ); 1.88 (1H, m, H-6 $\beta$ ); 1.74 (2H, m, CH<sub>2</sub>-7); 1.5 - 1.2 (6H, m, 3 x CH<sub>2</sub>); 1.11 (3H, s, CH<sub>3</sub>); 1.02 (3H, s, CH<sub>3</sub>).

### Example 23

**Preparation of 6'-bromo-6'-desoxy-homoharringtonine, from anhydro-homoharringtonine resulting from Example 21:**



To a stirred solution of product resulting from Example 21 (60 mg, 0.114 mmol)

in anhydrous dichloromethane (300  $\mu$ l) was added at  $-10^{\circ}\text{C}$  a commercial solution of hydrobromic acid in acetic acid (205  $\mu$ l, 1.02 mmol, HBr 30% w/w). After stirring at  $-10^{\circ}\text{C}$  for 3 hours, was added saturated sodium hydrogen carbonate solution up to pH 8. The resulting aqueous layer was extracted three times with dichloromethane and the combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a yellow oil (60 mg, 87%). The crude product thus obtained showed the following characteristics:

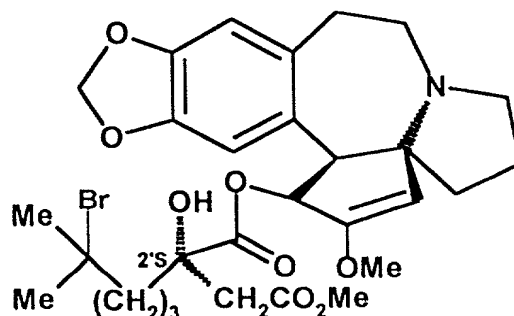
IR (ATR) ( $\text{cm}^{-1}$ ): 2957; 1744; 1653; 1487; 1223.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

6.63 (1H, s, H-17\*); 6.54 (1H, s, H-14\*); 5.99 (1H, d,  $J_{3-4} = 9.8$ , H-3); 5.87 (2H, m,  $\text{OCH}_2\text{O}$ ); 5.05 (1H, s, H-1); 3.78 (1H, d,  $J_{4-3} = 9.8$ , H-4); 3.69 (3H, s,  $\text{OCH}_3$ ); 3.58 (3H, s,  $\text{OCH}_3$ ); 3.54 (1H, s, 2'-OH); 3.10 (2H, m, H-11 $\beta$  + H-8 $\alpha$ ); 2.94 (1H, m, H-10 $\alpha$ ); 2.60 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.39 (1H, dd,  $J_{AB} = 14.0$ ,  $J = 6.8$ , H-11 $\alpha$ ); 2.26 and 1.89 (2H, 2d,  $J_{AB} = 16.5$ ,  $\text{CH}_2\text{CO}_2$ ); 2.03 (1H, m, H-6 $_A$ ); 1.91 (1H, m, H-6 $_B$ ); 1.75 (2H, m,  $\text{CH}_2$ -7); 1.74 (3H, s,  $\text{CH}_3$ ); 1.72 (3H, s,  $\text{CH}_3$ ); 1.6 - 1.2 (6H, m, 3 x  $\text{CH}_2$ ).

### Example 24

Preparation of 6'-bromo-6'-desoxy-epihomoharringtonine, from anhydro-epihomoharringtonine resulting from Example 22:



A commercial solution of hydrobromic acid in acetic acid (205  $\mu$ l, 1.02 mmol,

HBr 30% w/w) was added to a stirred solution of product resulting from Example 22 (60 mg, 0.114 mmol) in anhydrous dichloromethane (300  $\mu$ l) at  $-10^{\circ}\text{C}$ . After stirring at  $-10^{\circ}\text{C}$  for 3 hours, was added a saturated sodium hydrogen carbonate solution up to pH 8 and the resulting aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a yellow oil (63 mg, 91%). The crude product thus obtained showed the following characteristics:

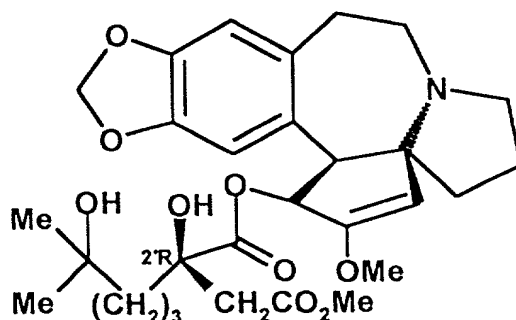
IR (ATR) ( $\text{cm}^{-1}$ ): 2957; 1744; 1653; 1487; 1223.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

6.64 (1H, s, H-17\*); 6.59 (1H, s, H-14\*); 5.97 and 5.87 (2H, 2d,  $J_{AB} = 1.1$ ,  $\text{OCH}_2\text{O}$ ); 5.95 (1H, d,  $J_{3-4} = 9.7$ , H-3); 5.04 (1H, s, H-1); 3.78 (1H, d,  $J_{4-3} = 9.7$ , H-4); 3.67 (3H, s,  $\text{OCH}_3$ ); 3.66 (3H, s,  $\text{OCH}_3$ ); 3.49 (1H, s, 2'-OH); 3.10 (2H, m, H-11 $\beta$  + H-8 $\alpha$ ); 2.93 (1H, m, H-10 $\alpha$ ); 2.62 and 2.54 (2H, 2d,  $J_{AB} = 16.5$ ,  $\text{CH}_2\text{CO}_2$ ); 2.60 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.40 (1H, m, H-11 $\alpha$ ); 2.03 (1H, m, H-6 $_A$ ); 1.89 (1H, m, H-6 $_B$ ); 1.74 (2H, m,  $\text{CH}_2$ -7); 1.72 (3H, s,  $\text{CH}_3$ ); 1.70 (3H, s,  $\text{CH}_3$ ); 1.6 - 0.7 (6H, m, 3 x  $\text{CH}_2$ ).

### Example 25

Preparation of homoharringtonine, from 6'-bromo-6'-desoxy-homoharringtonine resulting from Example 23



1) Method A:

A 5% sodium hydrogen carbonate solution (3 ml) was added to a stirred solution of product resulting from Example 23 (60 mg, 0.099 mmol) in acetone (1.5 ml). After stirring at room temperature for 2 hours, the reaction mixture was evaporated *in vacuo* and the residual aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (55 mg) was purified by column chromatography (dichloromethane, then dichloromethane / methanol (99:1 then 95:5), silica (15-40  $\mu\text{m}$ ) 2.75 g) to provide homoharringtonine (29 mg, 47%). The product thus obtained showed following characteristics:

$[\alpha]_{\text{D}}^{20}$ : -110 ( $c = 0.24$ ;  $\text{CHCl}_3$ )

IR (film NaCl) ( $\text{cm}^{-1}$ ): 3468; 2961; 1745; 1656; 1487; 1224; 1033.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

6.63 (1H, s, H-17\*); 6.55 (1H, s, H-14\*); 6.01 (1H, d,  $J_{3-4} = 9.8$ , H-3); 5.87 (2H, m,  $\text{OCH}_2\text{O}$ ); 5.05 (1H, s, H-1); 3.78 (1H, d,  $J_{4-3} = 9.8$ , H-4); 3.68 (3H, s,  $\text{OCH}_3$ ); 3.58 (3H, s,  $\text{OCH}_3$ ); 3.54 (1H, s, 2'-OH); 3.10 (2H, m, H-11 $\beta$  + H-8 $\alpha$ ); 2.95 (1H, m, H-10 $\alpha$ ); 2.59 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.38 (1H, dd,  $J_{\text{AB}} = 14.0$ ,  $J = 6.7$ , H-11 $\alpha$ ); 2.27 and 1.90 (2H, 2d,  $J_{\text{AB}} = 16.5$ ,  $\text{CH}_2\text{CO}_2$ ); 2.02 (1H, m, H-6 $\text{A}$ ); 1.90 (1H, m, H-6 $\text{B}$ ); 1.76 (2H, m,  $\text{CH}_2$ -7); 1.5 - 1.15 (6H, m, 3 x  $\text{CH}_2$ ); 1.30 (1H, s, 6'-OH); 1.19 (6H, 2s, 2 x  $\text{CH}_3$ ).

## 2) Method B:

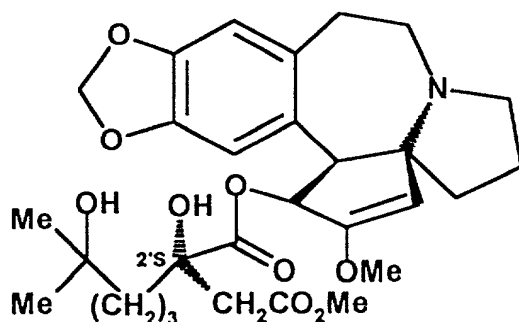
A saturated calcium carbonate solution (3 ml) was added to a stirred solution of product resulting from Example 23 (60 mg, 0.099 mmol) in acetone (3 ml). After stirring at room temperature for 2 hours, obtaining of product resulting from method A was specified by CCM.

## 3) Method C:

A saturated barium carbonate solution (9 ml) was added to a stirred solution of product resulting from Example 23 (60 mg, 0.099 mmol) in acetone (3 ml). After stirring at room temperature for 2 hours, obtaining of product resulting from method A was specified by CCM.

**4) Method D:**

To a stirred solution of product resulting from Example 23 (60 mg, 0.099 mmol) in a mixture acetone/water (3/2, 2.15 ml) was added silver nitrate (25 mg, 0.149 mmol). After stirring at room temperature for 2 hours, obtaining of product resulting from method A was specified by CCM.

**Example 26****Preparation of epihomoharringtonine:**

**1°) from 6'-bromo-6'-desoxy-epihomoharringtonine resulting from Example 24:**

**a) Method A:**

A 5% sodium hydrogen carbonate solution (3 ml) was added to a stirred solution of product resulting from Example 24 (60 mg, 0.099 mmol) in acetone (1.75 ml). After stirring at room temperature for 2 hours, the reaction mixture was evaporated *in vacuo* and the residual aqueous layer was extracted three times with dichloromethane. The combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (60 mg) was purified by column chromatography (dichloromethane, then dichloromethane / methanol (99:1 then 97:3), silica (15–40  $\mu$ m) 3 g) to provide epihomoharringtonine (29 mg, 47%). The product thus obtained showed following characteristics:

$[\alpha]_D^{20}$ : -92 (c = 0,29;  $\text{CHCl}_3$ )

IR (film NaCl) ( $\text{cm}^{-1}$ ): 3514; 2961; 1744; 1655; 1488; 1223; 1035.

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

6.65 (1H, s, H-17\*); 6.60 (1H, s, H-14\*); 5.95 (1H, d, H-3); 5.95 and 5.86 (2H, 2d, OCH<sub>2</sub>O); 5.05 (1H, s, H-1); 3.78 (1H, d, J<sub>4,3</sub> = 9.7, H-4); 3.68 (3H, s, OCH<sub>3</sub>);  
 5 3.66 (3H, s, OCH<sub>3</sub>); 3.52 (1H, br.s, 2'-OH); 3.13 (2H, m, H-11β + H-8α); 2.97 (1H, m, H-10α); 2.63 (2H, m, H-8β + H-10β); 2.61 and 2.52 (2H, 2d, J<sub>AB</sub> = 16.5, CH<sub>2</sub>CO<sub>2</sub>); 2.40 (1H, dd, J<sub>AB</sub> = 13.8, J = 6.3, H-11α); 2.04 (1H, m, H-6<sub>A</sub>); 1.94 (1H, m, H-6<sub>B</sub>); 1.78 (2H, m, CH<sub>2</sub>-7); 1.45 - 0.7 (6H, m, 3 x CH<sub>2</sub>); 1.16 (3H, s, CH<sub>3</sub>); 1.15 (3H, s, CH<sub>3</sub>).

10

**b) Method B:**

A saturated calcium carbonate solution (3 ml) was added to a stirred solution of product resulting from Example 24 (60 mg, 0.099 mmol) in acetone (3 ml). After stirring at room temperature for 2 hours, obtaining of product resulting  
 15 from method A was specified by CCM.

**c) Method C:**

A saturated barium carbonate solution (9 ml) was added to a stirred solution of product resulting from Example 24 (60 mg, 0.099 mmol) in acetone (3 ml). After  
 20 stirring at room temperature for 2 hours, obtaining of product resulting from method A was specified by CCM.

**d) Method D:**

To a stirred solution of product resulting from Example 24 (60 mg, 0.099 mmol) in a mixture acetone/water (3/2, 2.15 ml) was added silver nitrate (25 mg, 0.149 mmol). After stirring at room temperature for 2 hours, obtaining of product resulting from method A was specified by CCM.

25

**2°) from anhydroepihomoharringtonine resulting from Example 22**

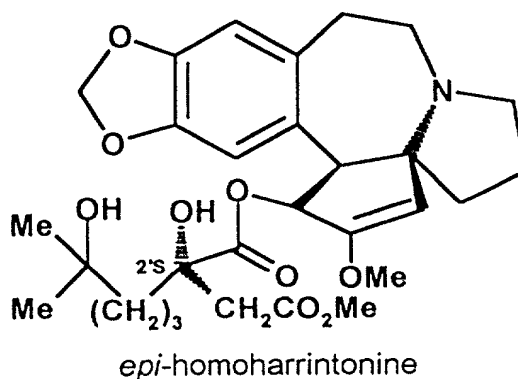
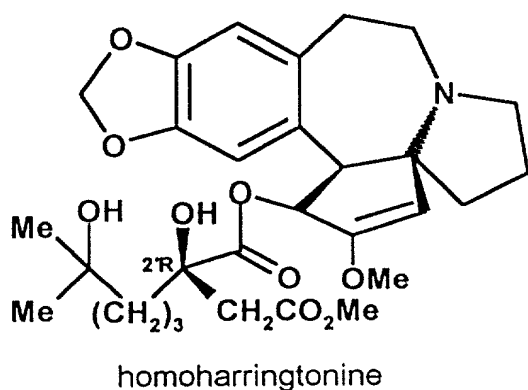
30

To a stirred solution of anhydroepihomoharringtonine resulting from Example 22 (58 mg, 0.109 mmol) in anhydrous dichloromethane (0.3 ml) was added at -10°C a commercial solution of hydrobromic acid in acetic acid (0.195 ml, 0.98 mmol, HBr 30% w/w). After stirring at -10°C for 3 hours, was added water (2.8  
 35 ml) and then the temperature was raised to 20°C. After stirring at 20°C for 3 hours, was added a sodium carbonate solution (0.76M; 6 ml) up to pH 8. The

resulting aqueous layer, after saturation with sodium chloride, was extracted with dichloromethane (3 x 10 ml) and the combined organic layers were dried over magnesium sulfate and evaporated to dryness to provide epihomoharringtonine (45 mg brut, 75%). The crude product thus obtained showed identical characteristics to this obtained with method A.

### Example 27

- 10 Preparation of homoharringtonine as a pharmaceutical use from crude semi-synthetic homoharringtonine resulting from Example 25 by preparative high-performance liquid chromatography:



15

#### 1°) Method A

- Crude homoharringtonine (35g) is dissolved in buffer (triethylamine (1.55 / 1000) in deionised water and orthophosphoric acid to adjust pH to 3. The solution was filtered then injected on a preparative high-performance liquid chromatograph equipped with axial compression and high pressure pump (stationary phase : n-octadecylsilane, 15  $\mu$ m, porosity 100, 1kg; mobile phase: buffer / tetrahydrofurane 85 / 15). Elution was performed at a flow rate of 0.2 l/min. Fractions contain was monitored by U.V. detector and TLC. Retained fraction were finally checked by HPLC then combined, alkalised with 2.5% aqueous ammonia and extracted with dichloromethane (4 x 400 ml), After concentration under reduced pressure homoharringtonine is obtained as a pale yellow resin which on trituration in a 8/2 water-methanol mixture gave pure homoharringtonine as a white crystalline solid (mp = 127°C), HPLC purity was



higher than 99.8%.

### 2°) Method B

Same procedure of purification as method A was performed but mobile phase  
5 buffer / methanol (68 /32) was used instead buffer / tetrahydrofurane.

### 3°) Method C

Same procedure of purification as method A was performed but mobile phase  
10 buffer / acetonitrile (85 /15) was used instead buffer / tetrahydrofurane.

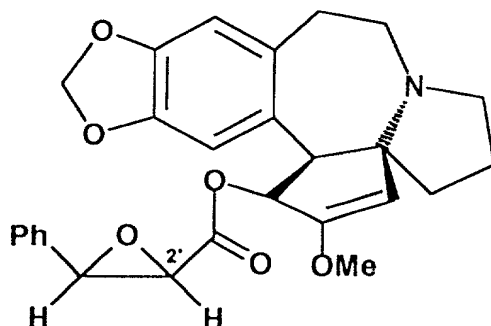
## Example 28

### Preparation of homoharringtonine as a pharmaceutical use from semi- 15 purified natural cephalotaxine

Crude homoharringtonine, prepared according to Example 25 from a partially  
racemized natural cephalotaxine and purified by chromatography and  
crystallisation according to the method A of Example 27, gave an  
20 homoharringtonine showing a non natural enantiomeric *epi*-homoharringtonine  
content less than 0.05%.

## Example 29

25 Preparation of 2'-de-(methoxycarbonylmethyl)-(2-O-3')-  
dehydroneoharringtonine or cephalotaxyl phenylglycidate, via  
esterification of cephalotaxine with phenylglycidic acid



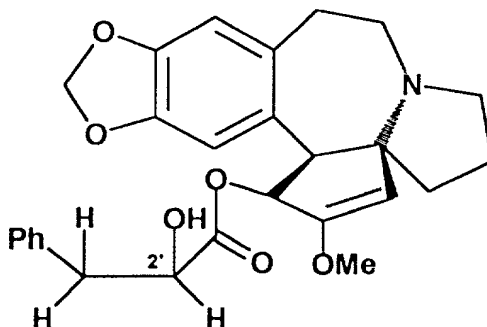
To a stirred mixture of (2R,3R)-cis-phenylglycidic acid (78 mg, 0.48 mmol) in anhydrous toluene (2 ml) was added 1,3-dicyclohexylcarbodiimide (130 mg, 0.63 mmol). After stirring for 10 minutes at room temperature, cephalotaxine (50 mg, 0.16 mmol) and pyrrolidinopyridine (24 mg, 0.16 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 15 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 8:2), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product (200 mg) was purified by column chromatography (dichloromethane / methanol (98:2), silica (15–40  $\mu\text{m}$ ) 4g) to provide expected product (19 mg, 27%). The product thus obtained showed following characteristics:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

7.27 (3H, m, Ph); 7.18 (2H, m, Ph); 6.63 (1H, s, H-17\*); 6.40 (1H, s, H-14\*); 5.96 and 5.85 (2H, 2d,  $J_{AB} = 1.5$ ,  $\text{OCH}_2\text{O}$ ); 5.73 (1H, d,  $J_{3-4} = 9.4$ , H-3); 5.01 (1H, s, H-1); 4.01 (1H, d,  $J_{3'-2'} = 4.6$ , H-3'); 3.65 (3H, s,  $\text{OCH}_3$ ); 3.62 (1H, d,  $J_{4-3} = 9.3$ , H-4); 3.40 (1H, d,  $J_{2'-3'} = 4.5$ , H-2'); 3.27 (1H, m,  $J_{AB} = 14.3$ ,  $J = 12.1$  and 7.8, H-11 $\beta$ ); 3.05 (1H, m, H-8 $\alpha$ ); 2.91 (1H, td,  $J = 11.7$  and 7.4, H-10 $\alpha$ ); 2.57 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.43 (1H, dd,  $J_{AB} = 14.5$ ,  $J = 7.0$ , H-11 $\alpha$ ); 1.93 (1H, m, H-6 $\alpha$ ); 1.84 (1H, m, H-6 $\beta$ ); 1.68 (2H, m,  $\text{CH}_2$ -7).

### Example 30

Preparation of 2'-de-(methoxycarbonylmethyl)-neoharringtonine, via hydrogenolysis of cephalotaxyl phenylglycidate resulting from Example

29

To a stirred solution of cephalotaxyl phenylglycidate resulting from Example 29 (200 mg, 0.433 mmol) in methanol (10 ml) was added 10% palladium on charcoal (100 mg). The resulting mixture was stirred at room temperature under hydrogen pressure (50 p.s.i.) for 4 hours, and the reaction mixture was filtered and evaporated to dryness. The resulting crude product (175 mg) was purified by column chromatography (dichloromethane / methanol (99:1 then 98:2), silica (15–40  $\mu$ m) 5.5 g) to provide an amber solid (86 mg, 43%). The product thus obtained showed following characteristics:

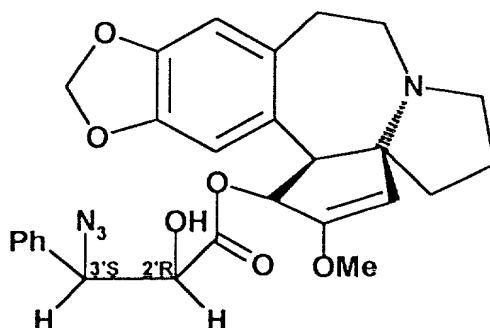
IR (pastille KBr) ( $\text{cm}^{-1}$ ): 3436; 2937; 1747; 1655; 1487; 1224 et 1035.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

7.25 (3H, m, *m,p*-Ph); 7.0 (2H, m, *o*-Ph); 6.65 (1H, s, H-17\*); 6.63 (1H, s, H-14\*); 5.98 (1H, d,  $J_{3-4} = 9.3$ , H-3); 5.85 (2H, 2d,  $J_{AB} = 1.2$ ,  $\text{OCH}_2\text{O}$ ); 5.09 (1H, s, H-1); 4.17 (1H, m, H-2'); 3.85 (1H, d,  $J_{4-3} = 9.6$ , H-4); 3.71 (3H, s,  $\text{OCH}_3$ ); 3.20 (1H, m, H-11 $\beta$ ); 3.10 (1H, m, H-8 $\alpha$ ); 2.95 (1H, m, H-10 $\alpha$ ); 2.60 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.39 (2H, m, H-11 $\alpha$  + H-3' $_A$ ); 2.04 (1H, m, H-6 $_A$ ); 2.0 (1H, dd,  $J_{AB} = 14.3$ ,  $J_{3'B-2'} = 9.5$ , H-3'B); 1.91 (1H, m, H-6 $_B$ ); 1.77 (2H, m,  $\text{CH}_2$ -7).

Example 31

Preparation of 2'R-de-(methoxycarbonylmethyl)-3'S-azido-neoharringtonine, from cephalotaxyl phenylglycidate resulting from Example 29



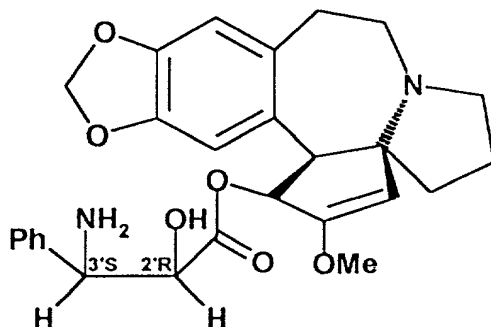
To a stirred solution of cephalotaxyl phenylglycidate resulting from Example 29 (100 mg, 0.217 mmol) in a mixture of methanol/water (8/1, 1.27 ml) was added sodium azide (70 mg, 1.08 mmol) and methyl formate (174  $\mu$ l, 2.82 mmol). After stirring at 50°C for 68 hours, and cooling at ambient temperature, was added 5% sodium hydrogen carbonate solution up to pH 8. The resulting aqueous layer was extracted three times with dichloromethane and the combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (120 mg) was purified by column chromatography (dichloromethane / methanol (99:1), silica (15-40  $\mu$ m) 3.5g) to provide a viscous yellow oil (84 mg, 76%). The product thus obtained showed following characteristics:

IR (ATR) ( $\text{cm}^{-1}$ ): 3488; 2935; 2105; 1748; 1654; 1486; 1223; 1034.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz): 7.38 (3H, m, *m,p*-Ph); 7.29 (2H, m, *o*-Ph); 6.74 (1H, s, H-17\*); 6.67 (1H, s, H-14\*); 6.08 (1H, d,  $J_{3-4} = 9.8$ , H-3); 5.90 (2H, 2d,  $J_{AB} = 1.4$ ,  $\text{OCH}_2\text{O}$ ); 5.08 (1H, s, H-1); 4.07 (1H, d large, H-2'); 3.85 (1H, d,  $J_{4-3} = 9.7$ , H-4); 3.78 (1H, br.s, H-3'); 3.69 (3H, s,  $\text{OCH}_3$ ); 3.23 (1H, m, H-11 $\beta$ ); 3.11 (1H, m, H-8 $\alpha$ ); 2.98 (1H, m, H-10 $\alpha$ ); 2.90 (1H, d,  $J_{2'-\text{OH}} = 8.2$ , 2'-OH); 2.63 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.47 (1H, dd,  $J_{AB} = 14.2$ ,  $J = 6.9$ , H-11 $\alpha$ ); 2.05 (1H, m, H-6 $_A$ ); 1.92 (1H, m, H-6 $_B$ ); 1.78 (2H, m,  $\text{CH}_2$ -7).

### Example 32

Preparation of 2'R-de-(methoxycarbonylmethyl)-3'S-amino-neoharringtonine, via hydrogenolysis of azide resulting from Example 31



To a stirred solution of product resulting from Example 29 (80 mg, 0.158 mmol) in a mixture ethyl acetate-methanol (9/1, 10 ml) was added 10% palladium on charcoal (40 mg). The resulting mixture was stirred at room temperature under hydrogen pressure (50 p.s.i.) for 15 hours and after CCM control the reaction mixture was filtered and evaporated to dryness to provide a white solid (67 mg, 88%). The crude product thus obtained showed following characteristics:

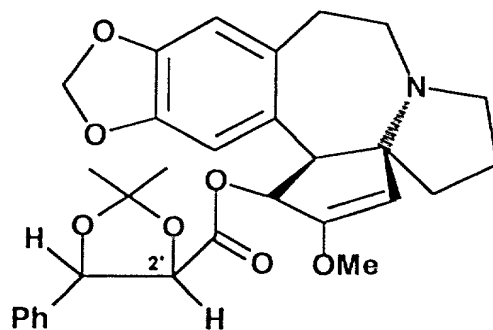
IR (ATR) ( $\text{cm}^{-1}$ ): 3299; 2935; 1740; 1654; 1486; 1222 et 1034.

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

7.27 (5H, m, Ph); 6.69 (1H, s, H-17\*); 6.67 (1H, s, H-14\*); 6.0 (1H, d,  $J_{3-4} = 9.7$ , H-3); 5.85 (2H, m,  $\text{OCH}_2\text{O}$ ); 5.09 (1H, br.s, H-1); 4.06 (1H, d,  $J = 1.2$ , H-2'); 3.86 (1H, d,  $J_{4-3} = 9.5$ , H-4); 3.72 (3H, s,  $\text{OCH}_3$ ); 3.38 (1H, br.s); 3.25 (1H, m, H-11 $\beta$ ); 3.14 (1H, m, H-8 $\alpha$ ); 2.99 (1H, m, H-10 $\alpha$ ); 2.64 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.49 (1H, m, H-11 $\alpha$ ); 2.05 (1H, m, H-6 $\alpha$ ); 1.94 (1H, m, H-6 $\beta$ ); 1.79 (2H, m,  $\text{CH}_2$ -7).

### Example 33

Preparation of acetone of 2'-de-(methoxycarbonylmethyl)-3'-hydroxy-neoharringtonine, via esterification of cephalotaxine

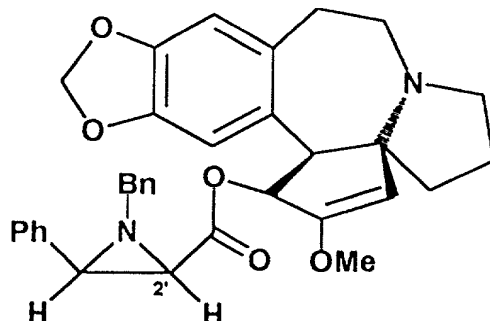


To a stirred mixture of isopropylidene-2,3-dihydroxy-3-phenylpropionic acid (17.5 mg, 0.078 mmol) in anhydrous toluene (1 ml) was added 1,3-dicyclohexylcarbodiimide (25 mg, 0.12 mmol). After stirring for 10 minutes at room temperature, cephalotaxine (75 mg, 0.24 mmol) and pyrrolidinopyridine (12 mg, 0.08 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 15 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 8:2), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product was purified by column chromatography (dichloromethane, then dichloromethane / methanol (98:2), silica (15–40  $\mu\text{m}$ )) to provide expected product (22 mg, 53%). The product thus obtained showed following characteristics:

- $^1\text{H}$  NMR** 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):
- 7.27 (5H, Ph); 6.63, 6.62, 6.60 and 6.57 (1H, 4s, H-14\*); 6.51, 6.49, 6.42 and 6.41 (1H, 4s, H-17\*); 5.93 ( $J_{3-4} = 9.6$ ), 5.89, 5.43 ( $J_{3-4} = 9.5$ ) and 5.31 ( $J_{3-4} = 9.3$ ) (1H, 4d, H-3); 5.89 (s), 5.87 + 5.84 (2d,  $J_{AB} = 1.5$ ), 5.85 + 5.80 (2d) and 5.84 + 5.77 (2d,  $J_{AB} = 1.5$ ) (2H,  $\text{OCH}_2\text{O}$ ); 5.23 ( $J_{5'-4'} = 7.3$ ), 5.20 ( $J_{5'-4'} = 7.4$ ), 4.58 ( $J_{5'-4'} = 8.0$ ) and 4.49 ( $J_{5'-4'} = 6.2$ ) (1H, 4d, H-5'); 5.07, 5.03 and 4.83 (1H, 3s, H-1); 4.32 ( $J_{4'-5'} = 7.4$ ), 4.21 ( $J_{4'-5'} = 6.2$ ), 4.18 ( $J_{4'-5'} = 7.4$ ) and 3.75 (1H, 4d, H-4'); 3.86 ( $J_{4-3} = 9.6$ ), 3.76 and 3.60 ( $J_{4-3} = 9.5$ ) (1H, 4d, H-4); 3.76, 3.75, 3.70 and 3.43 (3H, 4s,  $\text{OCH}_3$ ); 3.3 - 1.6 (10H, m); 1.66 + 1.41, 1.65 + 1.37, 1.51 + 1.44 and 1.47 + 1.22 (6H, 8s, 2 x  $\text{CH}_3$ ).

### Example 34

**Preparation of cephalotaxyl N-benzyl-3-phenyl-aziridine-1-carboxylate via esterification of cephalotaxine**



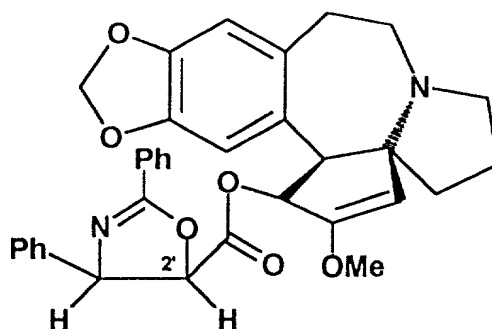
- 5 To a stirred mixture of (2S, 3S)- cis-N-benzyl-3-phenyl-aziridine-1-carboxylic acid (360 mg, 1.42 mmol) in anhydrous toluene (5 ml) was added 1,3-dicyclohexylcarbodiimide (390 mg, 1.9 mmol). After stirring for 5 minutes at room temperature, cephalotaxine (150 mg, 0.47 mmol) and pyrrolidinopyridine (70 mg, 0.47 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 2 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 8:2), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (15 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product (785 mg) was purified by column chromatography (dichloromethane, then dichloromethane / methanol (98:2), silica (15-40  $\mu\text{m}$ ) 23g) to provide a solid (240 mg, 92%). The product thus obtained showed following characteristics:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

- 7.24 (10H, m, 2xPh); 6.63 (1H, s, H-17\*); 6.60 (1H, s, H-14\*); 5.85 and 5.80 (2H, 2d,  $J_{\text{AB}} = 1.4$ ,  $\text{OCH}_2\text{O}$ ); 5.64 (1H, d,  $J_{3-4} = 9.3$ , H-3); 4.97 (1H, s, H-1); 3.92 and 3.20 (2H, 2d,  $J_{\text{AB}} = 13.7$ ,  $\text{CH}_2\text{Ph}$ ); 3.71 (1H, d,  $J_{4-3} = 9.4$ , H-4); 3.56 (3H, s,  $\text{OCH}_3$ ); 3.25 (1H, m, H-11 $\beta$ ); 3.07 (1H, m, H-8 $\alpha$ ); 2.93 (1H, m, H-10 $\alpha$ ); 2.86 (1H, d,  $J_{3-2'} = 6.8$ , H-3'); 2.57 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.38 (1H, dd,  $J_{\text{AB}} = 14.4$ ,  $J = 7.0$ , H-11 $\alpha$ ); 2.07 (1H, d,  $J_{2'-3'} = 6.8$ , H-2'); 1.96 (1H, m, H-6 $\text{A}$ ); 1.82 (1H, m, H-6 $\text{B}$ ); 1.70 (2H, m,  $\text{CH}_2$ -7).

**Example 35**

Preparation of N,O-anhydro-2'-de-(methoxycarbonylmethyl)-3'-benzamidoneo-harringtonine or cephalotaxyl N,O-anhydro-N-benzoyl-phenylisoserinate via esterification of cephalotaxine



5

To a stirred mixture of (4S, 5R)-2,4-diphenyl-4,5-dihydrooxazole-5-carboxylic acid (510 mg, 1.91 mmol) in anhydrous toluene (7 ml) was added 1,3-dicyclohexylcarbodiimide (525 mg, 2.54 mmol). After stirring for 15 minutes at room temperature, cephalotaxine (200 mg, 0.63 mmol) and pyrrolidinopyridine (95 mg, 0.64 mmol) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 3.5 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 9:1), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (15 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product (1 g) was purified by column chromatography (dichloromethane, then dichloromethane / methanol (98:2), silica (15–40  $\mu\text{m}$ )) to provide a yellow solid (330 mg, 91%). The product thus obtained showed following characteristics:

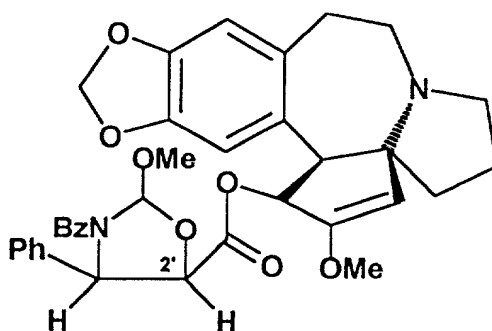
$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm, J Hz):

8.0 (2H, d, J = 7.3, o-PhC=N); 7.52 (1H, t, J = 7.4, p-PhC=N); 7.44 (2H, t, J = 7.5, m-PhC=N); 7.32 (2H, t, J = 7.2, m-Ph); 7.26 (1H, m, p-Ph); 7.15 (2H, d, J = 7.1, o-Ph); 6.58 (1H, s, H-17\*); 6.51 (1H, s, H-14\*); 5.98 (1H, d,  $J_{3-4} = 9.5$ , H-3); 5.85 and 5.76 (2H, 2d,  $J_{AB} = 1.3$ ,  $\text{OCH}_2\text{O}$ ); 5.08 (1H, s, H-1); 4.67 (1H, d,  $J_{4-5'} = 5.6$ , H-4'); 4.52 (1H, d,  $J_{5'-4'} = 5.6$ , H-5'); 3.85 (1H, d,  $J_{4-3} = 9.6$ , H-4); 3.70 (3H, s,  $\text{OCH}_3$ ); 3.17 (1H, m, H-11 $\beta$ ); 3.08 (1H, m, H-8 $\alpha$ ); 2.93 (1H, m, H-10 $\alpha$ ); 2.59 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.31 (1H, dd,  $J_{AB} = 14.2$ , J = 6.8, H-11 $\alpha$ ); 2.04 (1H, m, H-6 $\alpha$ ); 1.91 (1H, m, H-6 $\beta$ ); 1.75 (2H, m,  $\text{CH}_2$ -7).



Example 36

Preparation of N,O-methoxymethylene-2'-de-(methoxycarbonylmethyl)-3'-benz-amidoneoharringtonine or cephalotaxyl N,O-methoxymethylene-N-benzoyl-phenylisoserinate via esterification of cephalotaxine



To a stirred mixture of (4S, 5R)-3-N-benzoyl-2 *p*-methoxyphenyl-4-phenyloxazolidine-5-carboxylic acid (165 mg, 0.5 mmol) in anhydrous toluene (2 ml) was added 1,3-dicyclohexylcarbodiimide (140 mg, 0.68 mmol). After stirring for 5 minutes at room temperature, cephalotaxine (53 mg, 0.17 mmol) and pyrrolidinopyridine (25 mg, 0.17 mmol) were added. After stirring at 18 ± 5°C for 15 hours (with control of reaction in CCM, eluant dichloromethane / methanol; 9:1), the reaction mixture was filtered on ground-glass filter, the cake was washed with toluene (5 ml) and the filtrate was evaporated *in vacuo*. The resulting crude product (230 mg) was purified by column chromatography (dichloromethane, then dichloromethane / methanol (98:2), silica (15–40 μm) 7g) to provide expected product (90 mg, 86%). The product thus obtained showed following characteristics:

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

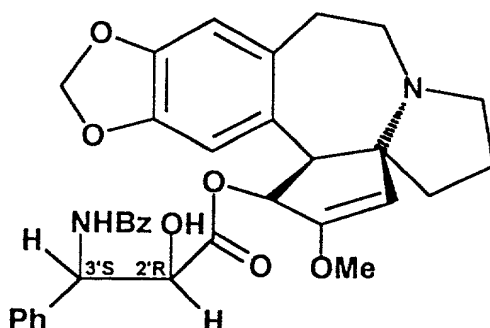
7.66 (2H, br.s, o-BzN); 7.41 (4H, m, BzN + Ph); 7.32 (2H, m, Ph); 7.26 (2H, m, Ph); 6.56 (1H, s, H-17\*); 6.54 (1H, s, H-14\*); 5.89 (1H, d, J<sub>3-4</sub> = 9.5, H-3); 5.83 and 5.80 (2H, 2m, OCH<sub>2</sub>O); 5.76 (1H, br.s, H-2'); 5.10 (1H, s, H-1); 4.85 (1H, br.s, H-4'); 4.42 (1H, br.s, H-5'); 3.84 (1H, d, J<sub>4-3</sub> = 9.5, H-4); 3.72 (3H, s, OCH<sub>3</sub>); 3.28 (3H, br.s, 2'-OCH<sub>3</sub>); 3.19 (1H, m, H-11β); 3.09 (1H, m, H-8α); 2.93 (1H, m, H-10α); 2.60 (2H, m, H-8β + H-10β); 2.37 (1H, dd, J<sub>AB</sub> = 14.4, J = 6.6,

H-11 $\alpha$ ); 2.03 (1H, m, H-6<sub>A</sub>); 1.90 (1H, m,  $J_{AB}$  = 12.2,  $J$  = 7.8 and 4.4, H-6<sub>B</sub>); 1.76 (2H, m, CH<sub>2</sub>-7).

5

### Example 37

#### Preparation of 2'-de-(methoxycarbonylmethyl)-3'-benzamido-neoharringtonine or cephalotaxyl N-benzoyl-phenylisoserinate



#### 10 1°) Via acidic hydrolysis of product resulting from Example 35

To a stirred solution of cephalotaxyl (4S, 5R)-2,4-diphenyl-4,5-dihydrooxazole-5-carboxylate resulting from Example 35 (300 mg, 0.53 mmol) in a mixture of methanol / tetrahydrofuran 50 / 50 (10 ml) was added at room temperature hydrochloric acid 1N (3.2 ml). After stirring at  $18 \pm 5^\circ\text{C}$  for 3 hours (with control of reaction in CCM), a saturated sodium hydrogen carbonate solution (19 ml) and a mixture of methanol / tetrahydrofuran 50 / 50 (50 ml) were added. After stirring at  $18 \pm 5^\circ\text{C}$  for 20 hours (with control of reaction in CCM), the reaction mixture was treated with ethyl acetate and water. The resulting aqueous layer was extracted with ethyl acetate and the combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (170 mg) was purified by column chromatography (dichloromethane / methanol (98:2), silica (15–40  $\mu\text{m}$ ) 8g) to provide a white solid (180 mg, 58%; HPLC purity 92.3%). The product thus obtained showed the following characteristics:

25

$[\alpha]_D^{20}$ : -119,2 ( $c$  = 0,141;  $\text{CHCl}_3$ )

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) (δ ppm, J Hz):

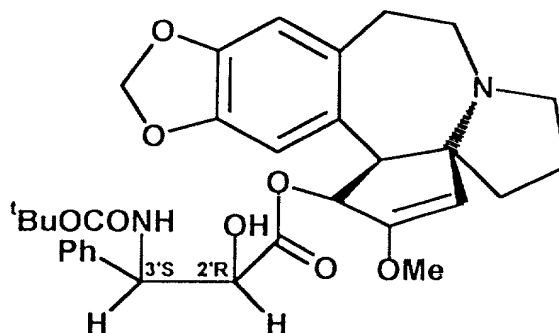
7.75 (2H, d, J = 7.3, *o*-BzN); 7.51 (1H, t, J = 7.3, *p*-BzN); 7.13 (2H, t, J = 7.3, *m*-BzN); Ph); 7.27 (5H, m, Ph); 6.88 (1H, d, J<sub>3'-NH</sub> = 7.9, 3'-NH); 6.59 (1H, s, H-17\*); 6.57 (1H, s, H-14\*); 5.93 (1H, d, J<sub>3-4</sub> = 9.7, H-3); 5.78 and 5.69 (2H, 2d, JAB = 1.5, OCH<sub>2</sub>O); 5.06 (1H, s, H-1); 4.98 (1H, dd, J<sub>3'-NH</sub> = 7.9, H-3'); 4.22 (1H, br.s, H-2'); 3.81 (1H, d, J<sub>4-3</sub> = 9.6, H-4); 3.58 (3H, s, OCH<sub>3</sub>); 3.19 (1H, m, J = 12.8, 7.9, H-11b); 3.07 (1H, m, H-8a); 2.93 (1H, m, H-10a); 2.72 (1H, br.s, 2'-OH), 2.58 (2H, m, H-8b + H-10b); 2.43 (1H, dd, J<sub>AB</sub> = 14.2, J = 7.0, H-11a); 2.01 (1H, m, H-6<sub>A</sub>); 1.88 (1H, m, J<sub>AB</sub> = 12.0, J = 7.8, 3.8, H-6<sub>B</sub>); 1.75 (2H, m, CH<sub>2</sub>-7).

## 2°) Via amidification of product resulting from Example 32

To a stirred solution of product resulting from Example 32 (60 mg, 0.125 mmol) in ethyl acetate (850 μl) were added a saturated sodium hydrogen carbonate solution (850 μl) and benzoyl chloride (19 μl, 0.163 mmol). A white precipitate was formed during the course of reaction. After stirring at room temperature for 1 hour, the reaction mixture was diluted with ethyl acetate and the organic layer was washed with a saturated sodium hydrogen carbonate solution. The resulting aqueous layer was extracted three times with ethyl acetate and the combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (65 mg) was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40 μm) 2.5 g) to provide a white solid (41 mg, 56%). The product thus obtained showed identical characteristics to this obtained from method above-mentioned.

## Example 38

Preparation of N-*ter*butoxycarbamoyl-2'-de-(methoxycarbonylmethyl)-3'-amino-neoharringtonine or cephalotaxyl N-*ter*butoxycarbamoyl-phenylisoserinate via amidification of product resulting from Example 32



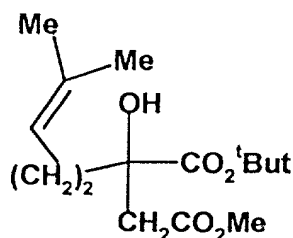
To a stirred solution of product resulting from Example 32 (60 mg, 0.125 mmol) in dichloromethane (850  $\mu$ l) were added a saturated sodium hydrogen carbonate solution (850  $\mu$ l) and di-tert-butyl dicarbonate (27 mg, 0.125 mmol). After stirring at room temperature for 1 hour, the reaction mixture was diluted with dichloromethane and the organic layer was washed with brine. The resulting aqueous layer was extracted three times with dichloromethane and the combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting crude product (70 mg) was purified by column chromatography (dichloromethane / methanol (98:2), silica (15-40  $\mu$ m) 3 g) to provide a white solid (40 mg, 55%). The product thus obtained showed the following characteristics:

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) ( ppm, J Hz):

7.27 (3H, m, *m,p*-Ph); 6.94 (2H, d, J = 6.6, *o*-Ph); 6.71 (1H, s, H-17\*); 6.66 (1H, s, H-14\*); 6.01 (1H, d, J<sub>3-4</sub> = 9.7, H-3); 5.90 (2H, s, OCH<sub>2</sub>O); 5.06 (1H, s, H-1); 5.05 (1H, m, NH); 4.56 (1H, m, H-3'); 4.15 (1H, m, H-2'); 3.81 (1H, d, J<sub>4-3</sub> = 9.7, H-4); 3.69 (3H, s, OCH<sub>3</sub>); 3.19 (1H, m, H-11 $\beta$ ); 3.10 (1H, m, H-8 $\alpha$ ); 2.93 (1H, m, H-10 $\alpha$ ); 2.61 (2H, m, H-8 $\beta$  + H-10 $\beta$ ); 2.51 (1H, m, H-11 $\alpha$ ); 2.05 (1H, m, H-6<sub>A</sub>); 1.89 (1H, m, H-6<sub>B</sub>); 1.77 (2H, m, CH<sub>2</sub>-7); 1.44 (9H, s, OC(CH<sub>3</sub>)<sub>3</sub>).

### Example 39

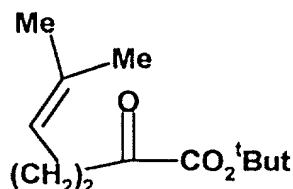
Preparation of tert-butyle 2-methoxycarbonylmethyl-2-hydroxy-6-methylhept-5-enoate:



### 1°) Preparation of intermediate oxalate

- 5 5-Bromo-2-methyl-pent-2-ene (1.34 g, 8.2 mmol) was added dropwise to a stirred mixture of magnesium (240 g, 10 mmol) (activated with further crystal of iodine) in anhydrous tetrahydrofuran (8 ml). The onset of the reaction is accompanied with a vigorous overheating and refluxing of the reaction mixture. The reflux was maintained until most of magnesium had reacted and the
- 10 reaction mixture was diluted with anhydrous tetrahydrofuran (16 ml). To a stirred mixture of tert-butyl ethyl oxalate (1.4 g, 8 mmol) in anhydrous tetrahydrofuran (8 ml) was added the resulting Grignard reagent at  $-78^{\circ}\text{C}$  over a period of 20 minutes. The temperature was allowed to rise to  $-15^{\circ}\text{C}$  over a period of 2 hours and the mixture was quenched with hydrochloric acid
- 15 1N. The separated organic layer was washed three times with brine, dried over magnesium sulfate and evaporated to dryness. The resulting crude product (2 g) was purified by column chromatography (cyclohexane / ethyl acetate (98:2), silica (15–40  $\mu\text{m}$ ) 80 g) to provide an oil (660 mg, 39 %). The intermediate  $\alpha$ -cetoester showed the following characteristics:

20



$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) (ppm, J Hz):

5.08 (1H, m, H-5); 2.80 (2H, t,  $J = 7.3$ ,  $\text{CH}_2$ -3); 2.28 (2H, m,  $\text{CH}_2$ -4); 1.68 (3H, s,  $\text{CH}_3$ ); 1.62 (3H, s,  $\text{CH}_3$ ); 1.54 (9H, s, O-tertBu).

## 2°) Preparation of the title compound

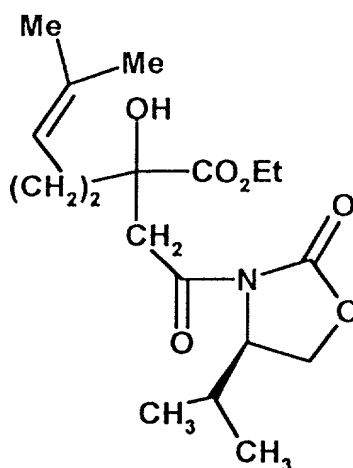
To a stirred solution of lithium bis-(trimethylsilylamide) 1M in tetrahydrofuran (9 ml, 9 mmol) was added anhydrous methyl acetate (0.7 ml, 8.75 mmol) at –78°C over a period of 1 minute and this was allowed the reaction to proceed at –78 ± 5°C for 20 minutes. To a stirred mixture of tert-butyl 2-oxo-6-methylhept-5-enoate prepared above (640 mg, 3 mmol) in anhydrous tetrahydrofuran (10 ml) was added the lithium enolate at –78°C over a period of 5 minutes and the resulting mixture was stirred at –78 ± 5°C for 30 minutes. After monitoring in CCM, the freezing bath was removed and the mixture was quenched with 15% ammonium chloride solution (10 ml). The separated organic layer was washed with 15% ammonium chloride solution (10 ml) and evaporated to dryness. The aqueous layers were extracted with ether (2 x 10 ml). The organic layers were combined with the concentrate and washed with brine (10 ml), dried over magnesium sulfate and evaporated to dryness. The resulting crude product (1.3 g) was purified by column chromatography (cyclohexane / ethyl acetate (95:5), silica (15–40 µm) 60 g) to provide an oil (222 mg, 26%). The product thus obtained showed the following characteristics:

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) ( ppm, J Hz):

5.07 (1H, m, HC=); 3.67 (3H, s, OCH<sub>3</sub>); 3.66 (1H, s, OH); 2.86 et 2.67 (2H, 2d, J<sub>AB</sub> = 15.8 CH<sub>2</sub>CO<sub>2</sub>); 2.13 (1H, m, CH<sub>2</sub>); 1.85 (1H, m, CH<sub>2</sub>); 1.67 (3H, s, CH<sub>3</sub>) et (2H, m, CH<sub>2</sub>); 1.59 (3H, s, CH<sub>3</sub>); 1.51 (9H, s, tert-BuO).

### Example 40

Preparation of ethyl 2-N-[(4'S)-isopropyl-2'-oxazolidino]carbonylmethyl-2-hydroxy-6-methylhept-5-enoate:



A commercial solution of lithium bis-(trimethylsilylamide) 1M in tetrahydrofuran (1.28 ml, 1.28 mmol) was added to a stirred solution of (4S)-3-acetyl-4-isopropyl-2-oxazolidinone (200 mg, 1.17 mmol) in anhydrous tetrahydrofuran at  $-78^{\circ}\text{C}$  and this was allowed the reaction to proceed at  $-78^{\circ}\text{C}$  for 30 minutes. Then a solution of ethyl 2-oxo-6-methylhept-5-enoate (323 mg, 1.75 mmol) in anhydrous tetrahydrofuran (5 ml) was added and the resulting mixture was stirred at  $-78^{\circ}\text{C}$  for 1 hour. After monitoring in CCM, the mixture was quenched with 15% ammonium chloride solution (5 ml). The separated organic layer was washed with 15% ammonium chloride solution (10 ml), then with brine (10 ml), dried over magnesium sulfate and evaporated to dryness. The  $^1\text{H}$  NMR spectra of the crude product showed a diastereomeric mixture  $\sim 2.5 / 1$ . The resulting crude product (516 mg) was purified by column chromatography (cyclohexane / ethyl acetate (90:10 to 80:20), silica (15-40  $\mu\text{m}$ ) 25 g) to provide the minority diastereomer like a yellow oil (55 mg, 13.7%) showing the following characteristics:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) (ppm, J Hz):

5.06 (1H, m, H-3'); 4.41 (1H, m, H-4''); 4.25 (4H, m,  $\text{CH}_2$ -5' and  $\text{OCH}_2\text{CH}_3$ ); 3.72 (1H, s, OH); 3.52 and 3.41 (2H, 2d,  $J_{\text{ax}} = 17.9$ ,  $\text{CH}_2$ -3); 2.36 (1H, m, H-6''); 2.16 (1H, m,  $\text{CH}_2$ ); 1.92 (1H, m,  $\text{CH}_2$ ); 1.75 (2H, m,  $\text{CH}_2$ ); 1.67 (3H, s,  $\text{CH}_3$ ); 1.59 (3H, s,  $\text{CH}_3$ ); 1.30 (3H, t,  $J = 7.1$ ,  $\text{OCH}_2\text{CH}_3$ ); 0.89 (3H, d,  $J = 7.0$ ,  $\text{CH}_3$ ); 0.87 (3H, d,  $J = 6.9$ ,  $\text{CH}_3$ ).

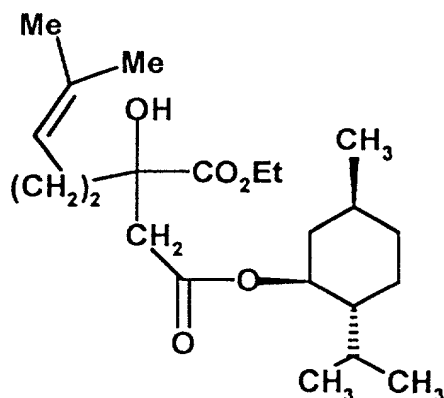
Then the majority diastereomer like a pale yellow oil (93 mg, 23.2%) showing the following characteristics:

<sup>1</sup>H NMR 400 MHz (CDCl<sub>3</sub>) ( ppm, J Hz):

5.06 (1H, m, H-3'); 4.40 (1H, m, H-4''); 4.23 (4H, m, CH<sub>2</sub>-5'' and OCH<sub>2</sub>CH<sub>3</sub>); 3.68 (1H, s, OH); 3.46 (2H, s, CH<sub>2</sub>-3); 2.33 (1H, m, H-6''); 2.16 (1H, m, CH<sub>2</sub>'); 1.91 (1H, m, CH<sub>2</sub>'); 1.75 (2H, m, CH<sub>2</sub>'); 1.67 (3H, s, CH<sub>3</sub>'); 1.59 (3H, s, CH<sub>3</sub>'); 1.28 (3H, t, J = 7.1 OCH<sub>2</sub>CH<sub>3</sub>); 0.90 (3H, d, J = 7.0, CH<sub>3</sub>''); 0.87 (3H, d, J = 6.9, CH<sub>3</sub>'').

### Example 41

#### 10 Preparation of ethyl 2-(1'R,2'S,5'R)-menthoxycarbonylmethyl-2-hydroxy-6-methylhept-5-enoate:



To a stirred commercial solution of lithium bis-(trimethylsilylamide) 1M in tetrahydrofurane (14 ml, 14 mmol), was added menthyl (1R,2S,5R)-(-)-acetate (2.8 g, 14.1 mmol) at -78°C and this was allowed the reaction to proceed at -78 °C for 30 minutes. To a stirred mixture of ethyl 2-oxo-6-methylhept-5-enoate prepared above (867 mg, 4.7 mmol) in anhydrous tetrahydrofurane (12 ml) was added the lithium enolate at -78°C over a period of 15 minutes and the resulting mixture was stirred at -78 °C for 3 minutes (CCM monitoring). The mixture was quenched with hydrochloric acid 1N (30 ml). The separated aqueous layer was extracted with *tert*-butyl methyl ether (2 x 15 ml) and the combined organic layers were washed with brine (3 x 15 ml), dried over magnesium sulfate and evaporated to dryness. The <sup>1</sup>H NMR spectra of the crude product showed a diastereomeric mixture ~6 / 4. The resulting crude product was purified by column chromatography (cyclohexane / ethyl acetate (98:2), silica (15-40 μm) 70 g) to provide the expected products (1 g, 57%). The separated diastereomers thus obtained showed the following characteristics:

Majority diastereomer:



$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) (ppm, J Hz):

5.03 (1H, t,  $J = 7.0$ , H-3'); 4.69 (1H, td,  $J = 10.9$  and  $4.3$ , H-1<sub>Men</sub>); 4.24 (2H, q,  $J = 7.0$ ,  $\text{OCH}_2\text{CH}_3$ ); 3.77 (1H, s, 2-OH); 2.91 and 2.67 (2H, 2d,  $J_{\text{ax}} = 16.4$ ,  $\text{CH}_2\text{CO}_2$ ); 2.13 (1H, m, CH'); 1.97 (1H, m, H-6eq<sub>Men</sub>); 1.85 (2H, m, CH' and H-7<sub>Men</sub>); 1.75-1.6 (4H, m, CH' and H-3eq, 4eq<sub>Men</sub>); 1.67 (3H, s, CH<sub>3</sub>); 1.58 (3H, s, CH<sub>3</sub>); 1.45 (1H, m, H-5<sub>Men</sub>); 1.35 (1H, m, H-2ax<sub>Men</sub>); 1.30 (3H, t,  $J = 6.9$ ,  $\text{OCH}_2\text{CH}_3$ ); 1.03 (1H, m, H-3ax<sub>Men</sub>); 0.93 (1H, m, H-6ax<sub>Men</sub>); 0.89 (6H, d,  $J = 6.8$ , 2 x CH<sub>3</sub>-Men); 0.87 (1H, m, H-4ax<sub>Men</sub>); 0.73 (3H, d,  $J = 6.9$ , CH<sub>3</sub>-Men).

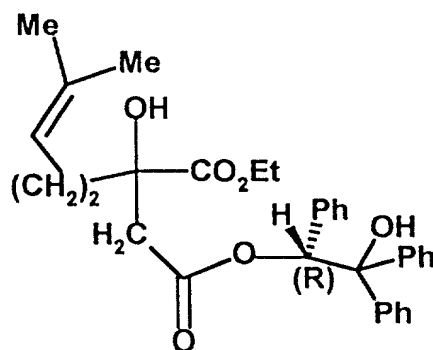
Minority diastereomer:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) (ppm, J Hz):

5.05 (1H, t,  $J = 7.0$ , H-3'); 4.67 (1H, td,  $J = 10.7$  and  $4.7$ , H-1<sub>Men</sub>); 4.25 (2H, m,  $\text{OCH}_2\text{CH}_3$ ); 3.74 (1H, s, 2-OH); 2.92 and 2.65 (2H, 2d,  $J_{\text{ax}} = 15.9$ ,  $\text{CH}_2\text{CO}_2$ ); 2.12 (1H, m, CH'); 1.97 (1H, m, H-6eq<sub>Men</sub>); 1.86 (2H, m, CH' and H-7<sub>Men</sub>); 1.75-1.6 (4H, m, CH' and H-3eq, 4eq<sub>Men</sub>); 1.67 (3H, s, CH<sub>3</sub>); 1.58 (3H, s, CH<sub>3</sub>); 1.48 (1H, m, H-5<sub>Men</sub>); 1.36 (1H, m, H-2ax<sub>Men</sub>); 1.31 (3H, t,  $J = 7.0$ ,  $\text{OCH}_2\text{CH}_3$ ); 1.15 - 0.8 (3H, m, H-3ax, 6ax, 4ax<sub>Men</sub>); 0.89 (6H, d,  $J = 6.9$ , 2 x CH<sub>3</sub>-Men); 0.76 (3H, d,  $J = 7.0$ , CH<sub>3</sub>-Men).

#### Example 42

Preparation of ethyl 2-[(R)-1',2',2'-triphenyl-ethan-2'-ol]ethoxycarbonylmethyl-2-hydroxy-6-methylhept-5-enoate:



A commercial solution of lithium bis-(trimethylsilylamide) 1M in tetrahydrofuran (6 ml, 6 mmol) was added to a stirred solution of R-(+)-1,1,2-triphenyl-1,2-ethanediol 2-acetate (665 mg, 2 mmol) in anhydrous tetrahydrofuran (6 ml) at  $-78^\circ\text{C}$ . The temperature was allowed to rise to  $0^\circ\text{C}$  over a period of 3 hour,

then was added anhydrous heptane (10 ml). To this stirred reaction mixture at  $-78^{\circ}\text{C}$  a solution of ethyl 2-oxo-6-methylhept-5-enoate (368 mg, 2 mmol) in anhydrous tetrahydrofuran (2 ml) was added and the temperature was allowed to rise to  $-40^{\circ}\text{C}$  over a period of 1 hour. After monitoring in CCM, the freezing bath was removed and the mixture was quenched with 15% ammonium chloride solution (10 ml). The separated organic layer was washed with 15% ammonium chloride solution (10 ml) and evaporated to dryness. The aqueous layers were extracted with dichloromethane (2 x 10 ml). The organic layers were combined with the concentrate and washed with brine (10 ml), dried over magnesium sulfate and evaporated to dryness. The  $^1\text{H}$  NMR spectra of the crude product showed a diastereomeric mixture ~60 / 40. The resulting crude product (820 mg) was purified by column chromatography (cyclohexane / ethyl acetate (97:3 to 95:5), silica (15-40  $\mu\text{m}$ ) 80 g) to provide the expected products (361 mg, 35%). The separated diastereomers thus obtained like white crystalline compounds showed the following characteristics:

Minority diastereomer:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) (ppm, J Hz):

7.66 (2H, d,  $J = 7.5$ , o-Ph); 7.43 (2H, t,  $J = 7.7$ , m-Ph); 7.35-7.0 (11H, m, Ph); 6.72 (1H, s, H-1"); 4.95 (1H, m, H-3"); 4.41 (2H, m,  $\text{OCH}_2\text{CH}_2$ ); 3.42 (1H, s, 2-OH); 2.90 and 2.67 (2H, 2d,  $J_{\text{ab}} = 16.5$ ,  $\text{CH}_2$ -3); 2.53 (1H, s, 2"-OH); 1.98 (1H, m,  $\text{CH}_2$ ); 1.8-1.5 (3H, m,  $\text{CH}_2$ ); 1.63 (3H, s,  $\text{CH}_3$ ); 1.52 (3H, s,  $\text{CH}_3$ ); 1.38 (3H, t,  $J = 7.1$ ,  $\text{OCH}_2\text{CH}_2$ ).

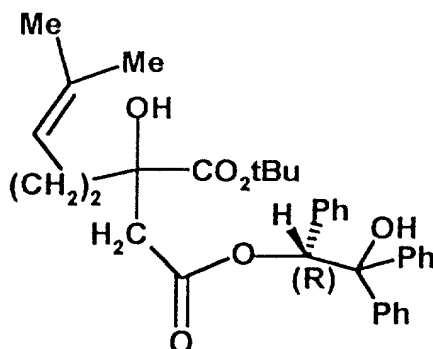
Majority diastereomer:

$^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) (ppm, J Hz):

7.52 (2H, d,  $J = 7.5$ , o-Ph); 7.36 (2H, t,  $J = 7.6$ , m-Ph); 7.27 (1H, t,  $J = 7.3$ , p-Ph); 7.2-7.0 (10H, m, Ph); 6.59 (1H, s, H-1"); 4.98 (1H, m, H-3"); 3.90 and 3.34 (2H, 2m,  $\text{OCH}_2\text{CH}_2$ ); 3.56 (1H, s, 2-OH); 3.22 (1H, s, 2"-OH); 2.88 and 2.69 (2H, 2d,  $J_{\text{ab}} = 16.7$ ,  $\text{CH}_2$ -3); 2.06 and 1.79 (2H, 2m,  $\text{CH}_2$ ); 1.7-1.5 (2H, m,  $\text{CH}_2$ ); 1.64 (3H, s,  $\text{CH}_3$ ); 1.54 (3H, s,  $\text{CH}_3$ ); 0.99 (3H, t,  $J = 7.1$ ,  $\text{OCH}_2\text{CH}_2$ ).

### Example 43

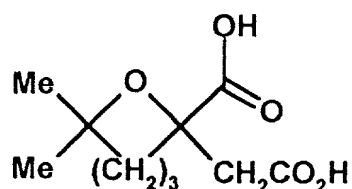
Preparation of tert-butyl 2-[(R)-1',2',2'-triphenyl-ethan-2'-ol]ethoxycarbonylmethyl-2-hydroxy-6-methylhept-5-enoate:



- 5 A commercial solution of lithium bis-(trimethylsilylamide) 1M in tetrahydrofuran (3 ml, 3 mmol) was added to a stirred solution of R-(+)-1,1,2-triphenyl-1,2-ethanediol 2-acetate (330 mg, 1 mmol) in anhydrous tetrahydrofuran (3.5 ml) at  $-78^{\circ}\text{C}$ . The temperature was allowed to rise to  $-10^{\circ}\text{C}$  over a period of 3 hour then was added anhydrous heptane (5 ml). To this stirred reaction mixture at
- 10  $-78^{\circ}\text{C}$  a solution of tert-butyl 2-oxo-6-methylhept-5-enoate (276 mg, 1.5 mmol) in anhydrous tetrahydrofuran (2 ml) was added and the temperature was allowed to rise to  $-40^{\circ}\text{C}$  over a period of 1 hour. After monitoring in CCM, the freezing bath was removed and the mixture was quenched with 15% ammonium chloride solution (5 ml). The separated organic layer was washed with 15%
- 15 ammonium chloride solution (5 ml) and evaporated to dryness. The aqueous layers were extracted with dichloromethane (2 x 10 ml). The organic layers were combined with the concentrate and washed with brine (5 ml), dried over magnesium sulfate and evaporated to dryness. The  $^1\text{H}$  NMR spectra of the crude product showed a diastereomeric mixture ~75 / 25. The resulting crude
- 20 product (550 mg) was purified by column chromatography (cyclohexane / ethyl acetate (96:4, 95/5 then 90:10), silica (15-40  $\mu\text{m}$ ) 60 g) to provide the expected products (217 mg, 40%). The majority diastereomer thus obtained like white crystalline compound showed the following characteristics:
- 25  $^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( ppm, J Hz):  
 7.53 (2H, d, J = 7.4, o-Ph); 7.36 (2H, t, J = 7.6, m-Ph); 7.28 (1H, t, J = 7.3, p-Ph); 7.2-7.0 (10H, m, Ph); 6.66 (1H, s, H-1''); 5.00 (1H, m, H-3'); 3.50 (1H, s, 2-OH); 2.94 (1H, s, 2'-OH); 2.76 and 2.61 (2H, 2d,  $J_{\text{AB}} = 16.3$ ,  $\text{CH}_2\text{-CO}_2$ ); 2.06 and 1.78 (2H, 2m,  $\text{CH}_2$ ); 1.65 (3H, s,  $\text{CH}_3$ ); 1.55 (3H, s,  $\text{CH}_3$  and 2H, m,  $\text{CH}_2$ ); 1.23 (9H, s, tert-BuO).
- 30

Example 44

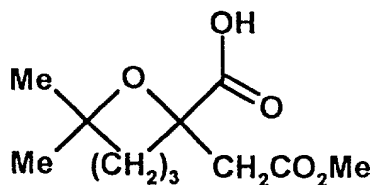
- 5 Preparation of 2-carboxymethyl-6,6-dimethyl-2-tetrahydro-  
pyranecarboxylic acid or O-demethylanhydrohomoharringtonic acid:

**(Method D)**

- 10 A solution of ethylenic diacid resulting from the Example 3 (1.5 g, 6.94 mmol)  
 in formic acid (2.6 ml) was stirred at 60°C for 16 hours. After return at ambient  
 temperature, formic acid was removal *in vacuo* and the resulting crude product  
 was dried at 40°C *in vacuo* for 20 hours (1.5 g, 100%).

- 15 Example 45

Preparation of 2-methoxycarbonylmethyl-6,6-dimethyl-2-tetrahydro-  
pyranecarboxylic or anhydrohomoharringtonic acid from product  
resulting from Example 39:



20

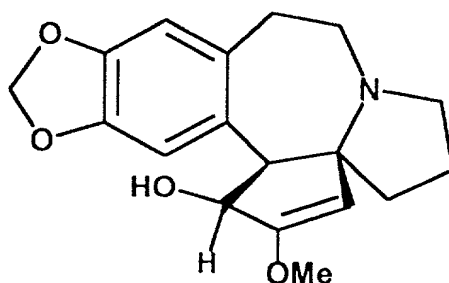
- A solution of tert-butyl 2-methoxycarbonylmethyl-2-hydroxy-6-methylhept-  
 enoate resulting from Example 39 (50 mg, 0.175 mmol) in formic acid (0.5 ml)  
 was stirred at room temperature for 9 days. After removal of formic acid *in*  
 25 *vacuo*, the residue was treated with 5% sodium hydrogen carbonate solution  
 up to pH 8. The aqueous layer was washed with ethyl acetate then, after

acidification (pH 1) with hydrochloric acid 1N, was extracted with ethyl acetate. The organic layer was dried over magnesium sulfate and evaporated to dryness to provide anhydrohomoharringtonic acid (20 mg, 50%).

5

#### Example 46

#### Preparation of purified (-) cephalotaxine from total alkaloidic extract of *Cephalotaxus sp*



10

Partially racemized cephalotaxine [H. Wenkui; L. Yulin; P. Xinfu, Scientia Sinica,; 23; 7; 835 (1980)]

15  $^1\text{H}$  NMR of two batches of cephalotaxine (extracted in the same conditions as above) with the optically active NMR shift reagent europium(III) *tris*[3-(heptafluoropropylhydroxymethylene)-(+)-camphorate (1 éq) showed the following results:

20 **Batch A:**  $^1\text{H}$  NMR 400 MHz ( $\text{CDCl}_3$ ) ( $\delta$  ppm): 6.06 (1H,  $\text{OCH}_2\text{O}$  (+)-cephalotaxine) and 5.82 (1H,  $\text{OCH}_2\text{O}$  (+)-cephalotaxine); 5.99 (1H,  $\text{OCH}_2\text{O}$  (-)-cephalotaxine) and 5.76 (1H,  $\text{OCH}_2\text{O}$  (-)-cephalotaxine).

Presence of  $11 \pm 5$  % de (+)-cephalotaxine.

$[\alpha]^{22} = -134,0^\circ$  ( $c = 0,214$ ;  $\text{CHCl}_3$ ) : calculated rate  $25 \pm 5$  %

25

**Batch B:** slightly racemized (1%)

$[\alpha]^{19} = -173,3^\circ$  ( $c = 0,208$ ;  $\text{CHCl}_3$ )

#### **Enantiomeric enrichment of the natural cephalotaxine:**

30 Crude chromatographed cephalotaxine (20g) was dissolved at  $55^\circ\text{C}$  in dry

methanol (100 ml). Crystallization occurs by cooling with rotary evaporator and after filtration the product thus obtained showed 99.9% of HPLC purity,  $[\alpha]^{20}_D = -130^\circ$  (C1, CHD<sub>3</sub>) corresponding to 10 % of racemization. The crystallized product thus obtained (20g) was dissolved again in hot methanol (100 ml).

Slowly cooling the solution allows translucent prisms composed of pure enantiomeric (-)-cephalotaxine  $[\alpha]^{20}_D = -185^\circ$  (C1, CHCl<sub>3</sub>).

After filtration, the mother liquors was allowed to slowly evaporate at room temperature and crystals in the form of macled needles exclusively composed of racemic cephalotaxine  $[\alpha]^{20}_D = 0,5^\circ$  (C1 ; CHCl<sub>3</sub>) were obtained.

After filtration, the second mother liquors allowed prisms composed of (-)-cephalotaxine identical to this obtained at the first crystallization.

After filtration, the third mother liquors still allowed macled needles (urchins) composed of (±)-cephalotaxine.

The cycle is repeated three times. The combined prismatic crystals was recrystallized once to give enantiomerically pure (-)-cephalotaxine, while the combined macled needles treated in the same way gives 100% racemic cephalotaxine.

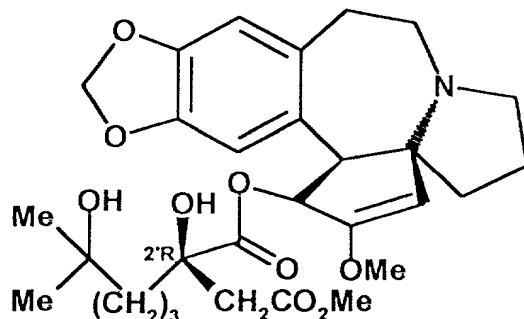
#### **Chemical evaluation of the enantiomeric purity of natural cephalotaxine:**

A sample of partially racemized natural cephalotaxine was inserted in the process, which sequence is described in the Examples 1,2,3,4,5,6,15,19 and 21, by using a pure (2R)-homoharrintonic acid resulting from Example 19.

The HPLC analysis of the diastereomeric mixture of anhydro-homoharrintonine thus obtained showed a significant enantio-*epi*-homoharringtonine rate (11% ± 3%) corresponding to the (+)-cephalotaxine content in the racemic mixture of origin (it has been demonstrated that the two antipodes of the homoharringtonic acid react in a stoichiometric way comparable to the pure enantiomeric cephalotaxine).

### **Example 47**

#### **Preparation of homoharringtonine, from anhydro-homoharringtonine:**



### 1° Method A

A commercial solution of hydrobromic acid in acetic acid (17.4 ml, 86.6 mmol, HBr 30% w/w) was added to a stirred solution of anhydrohomoharringtonine resulting from Example 21 (50.8 g, 9.63 mmol) in anhydrous dichloromethane (25.6 ml) at  $-10^{\circ}\text{C}$ . After stirring at  $-10^{\circ}\text{C}$  for 3 hours was added water (240 ml) and the reaction mixture was become viscous. The temperature was allowed to rise to room temperature and after stirring for 2.5 hours was added sodium carbonate 0.76M (406 ml) to pH 8. The resulting aqueous layer was saturated with sodium chloride, then was extracted with dichloromethane ( $3 \times 230$  ml) and the combined organic layers were dried over magnesium sulfate and evaporated to dryness to afford a foam. After phase reverse chromatography below-mentioned were obtained 4.03g of homoharringtonine (77%). The product thus obtained showed identical characteristics to this resulting from Example 25.

### 2° Method B

To a stirred solution of anhydrohomoharringtonine resulting from Example 21 (214 mg, 0.406 mmol) in anhydrous dichloromethane (1.1 ml) was added at  $-10^{\circ}\text{C}$  a commercial solution of hydrobromic acid in acetic acid (0.728 ml, 3.6 mmol, HBr 30% w/w). After stirring at  $-10^{\circ}\text{C}$  for 3 hours, was added water (13 ml) and then the temperature was raised to  $20^{\circ}\text{C}$ . After stirring at  $20^{\circ}\text{C}$  for 3 hours, was added a sodium carbonate solution (0.76M; 31.5 ml) up to pH 8. The resulting aqueous layer, after saturation with sodium chloride, was extracted with dichloromethane ( $3 \times 20$  ml) and the combined organic layers were dried over magnesium sulfate and evaporated to dryness. The resulting

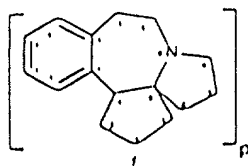
crude product was purified by phase reverse chromatography below-mentioned to provide homoharringtonine (166 mg, 75%). The product thus obtained showed identical characteristics to this resulting from Example 25.

09270006 031619

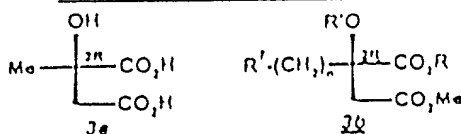


## DESCRIPTION AND DEFINITION OF HARRINGTONINES

## CEPHALOTAXANES

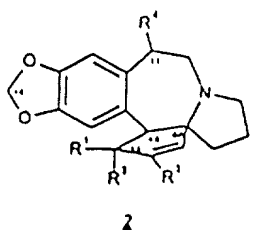


EXAMPLES OF HARRINGTONIC ACIDS (R=H), 3 AND OF  
HARRINGTONINES (R = CTX), 4



Citramalic acid Harringtonic acids R=H

## CEPHALOTAXANES



Examples of  
cephalotaxines:

Cephalotaxine =  
CTXOH 2a

R<sup>1</sup>=OH, R<sup>2</sup>=R<sup>4</sup>=H,  
R<sup>3</sup>=OMe;

Acetylcephalo-  
taxine, 2b

R<sup>1</sup>=Ac, R<sup>2</sup>=R<sup>4</sup>=H,  
R<sup>3</sup>=OMe

Cephalotaxi-  
none, 2c

R<sup>1</sup>, R<sup>2</sup>=O, R<sup>3</sup>=OMe,  
R<sup>4</sup>=H;

Demethylcepha-  
lotaxine, 2d

R<sup>1</sup>=OH, R<sup>2</sup>=R<sup>4</sup>=H,  
R<sup>3</sup>=OH;

Demethyl-  
cephalo-  
taxinone, 2e

R<sup>1</sup>, R<sup>2</sup>=O, R<sup>3</sup>=OH,  
R<sup>4</sup>=H;

11-Hydroxy-  
cephalo-  
taxinone, 2f

= 2b + R<sup>4</sup> = OH;

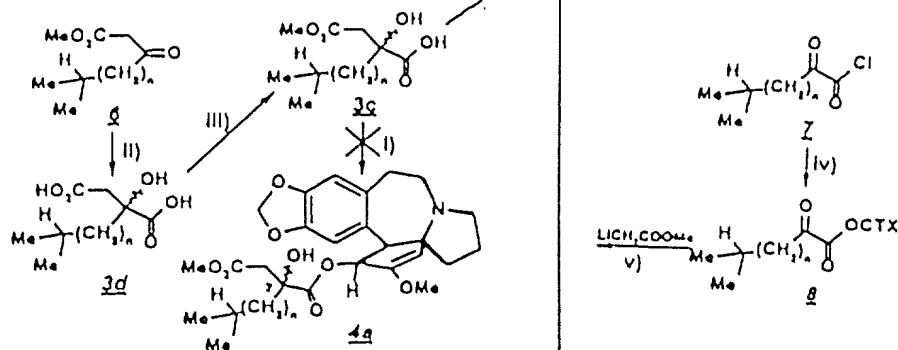
EXAMPLES OF HARRINGTONIC ACIDS  
(not isolated in the natural form as such)

R'	R'	n	R	
H	(CH <sub>2</sub> ) <sub>2</sub> C(OH)-	2	H	harringtonic acid
H	(CH <sub>2</sub> ) <sub>2</sub> C(OH)-	3	H	homoharringtonic acid
H	(CH <sub>2</sub> ) <sub>2</sub> C(OH)-	1	H	norharringtonic acid
H	(CH <sub>2</sub> ) <sub>2</sub> CH-	2	H	deoxyharringtonic acid
H	(CH <sub>2</sub> ) <sub>2</sub> CH-	3	H	anhydro-homo-harringtonic acid
H	Ph-	1	H	neoharringtonic acid

EXAMPLES OF HARRINGTONINES (natural)

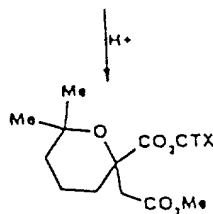
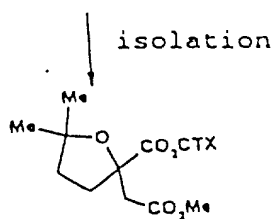
H	(CH <sub>2</sub> ) <sub>2</sub> C(OH)-	2	CTX	harringtonine: HT
H	(CH <sub>2</sub> ) <sub>2</sub> C(OH)-	3	CTX	homoharringtonine: HHT
H	(CH <sub>2</sub> ) <sub>2</sub> C(OH)-	1	CTX	norharringtonine
H	(CH <sub>2</sub> ) <sub>2</sub> CH-	2	CTX	deoxyharringtonine
H	(CH <sub>2</sub> ) <sub>2</sub> CH-	1	CTX	nordeoxyharringtonine
H	(CH <sub>2</sub> ) <sub>2</sub> CH-	3	CTX	homodeoxyharringtonine
H	(CH <sub>2</sub> ) <sub>2</sub> CH-	4	CTX	bishomodeoxyharringtonine
	-C(CH <sub>3</sub> ) <sub>2</sub> -O-	2	CTX	anhydroharringtonine
H	Ph-	1	CTX	neoharringtonine
H	Ph-	2	CTX	homo-neoharringtonine

Chemical reaction scheme showing the conversion of compound **2a** to compound **5**. Compound **2a** is a complex polycyclic molecule with a hydroxyl group and a methoxy group. It is converted to compound **5**, which has a different substitution pattern, including a methoxy group and a chlorine atom. The reaction is catalyzed by  $\text{Pd(PPh}_3)_4$  and  $\text{CuI}$  in toluene at  $100^\circ\text{C}$ .



i) Impossible on account of the steric hindrance [K.L. Mikolajczak et al., Tetrahedron, p.1995, (1972)]; ii) HCN, H<sup>+</sup>; iii) MeOH, H<sup>+</sup>; i') Silver salt of 3c [K.L. Mikolajczak et al., J. Med. Chem., p.63 (1975)]; iv) 2a/pyridine-dichloromethane; v) according to (T.R. Kelly et al., Tetrahedron Lett., 3501 (1973));

Natural homo-harringtonine



Anhydro-homo-harringtonine

[D.Z. Wang et al., Yaoxue Xuebao, p. 178, (1992)]

CLAIMS

What is claimed is:

1. A process for the preparation of sidechain-bearing cephalotaxane of the  
 5 following formula and/or a salt thereof

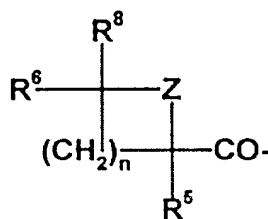


where

$\Omega$  ("omega") is a representative radical of the chain terminal moiety  
 and  $\text{-CO-}$  is the carbonyl of the ester group bonded to  
 cephalotaxane;

the  $\Omega\text{-CO-}$  radical is corresponding:

- either to the following substituted heterocycloalkane formula:



where n is included between 0 and 8;

Z is oxygen, nitrogen or sulfur heteroatom;

$\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are independently

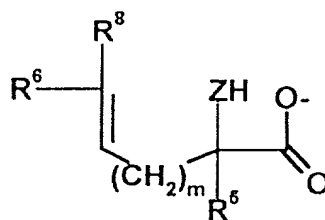
hydrogen;

hydrocarbon radical, saturated, insaturated or aromatic, linear or  
 ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl,

cycloalkenyl, aryl, heterocycloalkyl, of said radical including or not  
 heteroatom(s);  $\text{R}^6$  and  $\text{R}^8$  may be included in a cycle ;

oxygen ether bearing one of the former radicals;

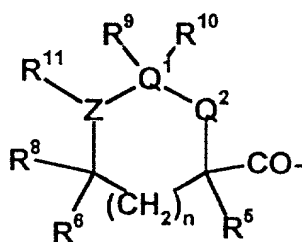
- or to the following linear alkene formula:



where m is included between 1 and 8;

$R^5$ ,  $R^6$  and  $R^8$  are as defined above;

- or to the following formula:



where n,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above;

Z and  $Q^2$  are independently oxygen, nitrogen or sulfur heteroatom;

$Q^1$  is carbon, silicon or phosphorus atom;

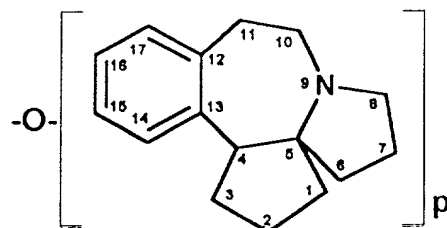
$R^9$  and  $R^{10}$  are independently hydrogen, alkoxy, hydrocarbon radical, including or not heteroatom(s), saturated, unsaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl;

$R^9$  and/or  $R^{10}$  having the ability to be null or taken together to make an heteroatom and/or make a multiple bond with  $Q^1$ ,  $R^9$  and  $R^{11}$  having the ability to be null to make a multiple bond between the two atoms of carbon bearing them; and

$R^{11}$  is hydrogen, arylcarbonyl, alkoxy carbonyl, aryloxy carbonyl or alkylcarbonyl;

where

-O-CTX is cephalotaxine moiety of the following formula a salt thereof:

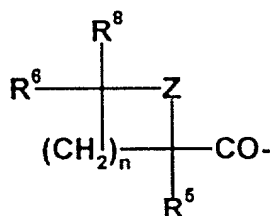


where p is equal to 1 or 2;

the two types of radicals  $-\Omega$  and  $-\text{CTX}$  above-mentioned being bonded with an ester bond  $-\text{CO}-\text{O}-$

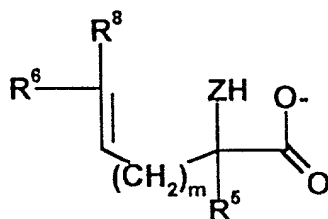
the said process bringing together:

- either carboxylic acid with general formula  $\Omega-\text{CO}-\text{OH}$  or a salt thereof;
- or an activated form of an acid with general formula  $\Omega-\text{CO}-\text{A}$  or a salt thereof, with  $\Omega-\text{CO}$  of the following formula:



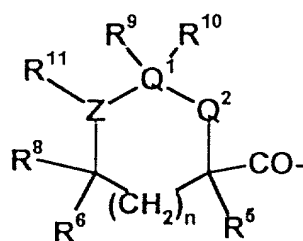
where n, Z,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined above;

where  $\Omega-\text{CO}$  of the following formula:



m is included between 1 and 8, Z,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined above;

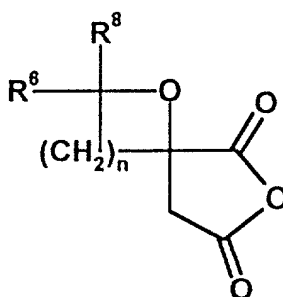
where  $\Omega-\text{CO}$  of the following formula:



where  $n$ ,  $Z$ ,  $Q^1$ ,  $Q^2$ ,  $R^5$ ,  $R^6$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  are as defined above

A represents:

- either cyclic anhydride of the following formula:



5

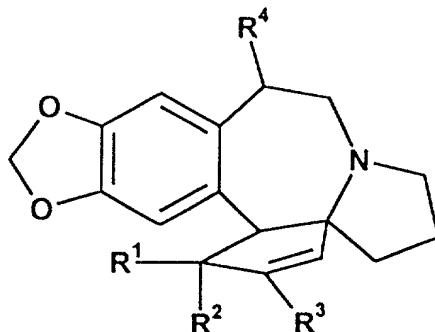
where  $n$ ,  $R^6$  and  $R^8$  are as defined above;

this reaction has been completed by methylation of the primary carboxyl thus formed, with:

- either a hydroxyl group bearing cephalotaxane or a salt thereof of the formula H-O-CTX, where CTX are as defined above;
  - or a metallic alcoxide of the formula M-O-CTX, where CTX are as defined above and M is a metal;
  - or an activated form of its hydroxyl group of the formula Y-O-CTX, where -O-CTX is as defined above and Y is, either a leaving group to allow a negative charge on oxygen atom by cleavage between Y- and -O-CTX, or to allow a carbocation by cleavage between Y-O- and -CTX;
- with the possible presence of one or several reaction additives to form said sidechain-bearing cephalotaxane and/or a salt thereof.

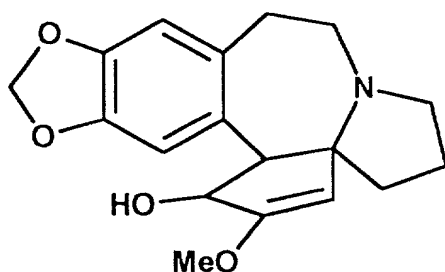
- 20 2. The process according to claim 1, wherein Z is an oxygen atom and the cephalotaxane H-O-CTX is a cephalotaxine of the following formula, or a salt

2. The process according to claim 1, wherein Z is an oxygen atom and the cephalotaxane H-O-CTX is a cephalotaxine of the following formula, or a salt thereof:



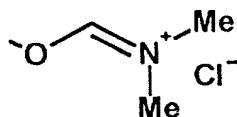
5 where  $R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are independently hydrogen, hydroxyl group or alkoxide.

3. The process according to claim 2, wherein said cephalotaxane H-O-CTX is cephalotaxine, or a salt thereof, where  $R^1$  is hydroxyl,  $R^2$  is methoxyl,  $R^3$  and  $R^4$  are hydrogen.



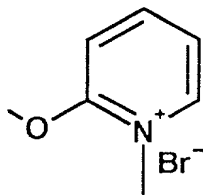
4. The process according to anyone of claims 1 to 3, wherein  $R^5$  is hydrogen.
5. The process according to anyone of claims 1 to 3, wherein  $R^5$  is  $-\text{CH}_2\text{-CO-O-Me}$ .
6. The process according to anyone of claims 1 to 5, wherein  $n = 1$  to 4,  $R^6$  and  $R^8$  are methyl.

7. The process according to anyone of claims 1 to 5, wherein  $n = 1$  or 2,  $R^6$  is phenyl and  $R^8$  is hydrogen.
- 5 8. The process according to claim 1 wherein,  $R^5$  is  $-\text{CH}_2\text{-CO-O-Me}$ , said cephalotaxane is the former of claim 3,  $n = 0$ , Z is a nitrogen atom and  $R^8$  is hydrogen.
9. The process according to anyone of claims 1 to 8, wherein A is  $\Omega\text{-CO-O-}$  radical where  $\Omega$  is as defined according to claim 1.
- 10 10. The process according to anyone of claims 1 to 8, wherein A is halide.
11. The process according to anyone of claims 1 to 8, wherein A is a radical of
- 15 compound  $\Omega\text{-CO-A}$  having the ability to generate cleavage of the bond between carbonyl group and substituent A of  $\Omega\text{-CO-A}$  to provide  $\Omega\text{-CO}^+$  and  $A^-$
12. The process according to anyone of claims 1 to 8, wherein A is a radical selected from substituents:
- 20 méthoxyformyloxy of formula  $\text{MeOCOO-}$ ,  
 trifluoroacétyloxy of formula  $\text{CF}_3\text{COO-}$ ,  
 alkylsulfonyloxy of formula  $\text{RSO}_3^-$ ,  
 phosphoxy of formula  $(\text{RO})_2\text{PO-}$ ,  
 halophosphoxy of formula  $\text{ROP}(\text{Cl})\text{O-}$ ,  
 25 trialkylsilyloxy of formula  $\text{R}_3\text{SiO-}$ ,  
 diméthyl-formamidineum chloride of formula:



or acyloxy-pyridinium bromide of formula:



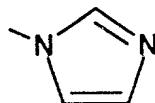


formulas wherein R is alkyl.

- 5 13. The process according to anyone of claims 1 to 8, wherein A is 2,4,6-trichlorobenzoyloxy radical

14. The process according to claim 13, wherein reagent of formula  $\Omega$ -CO-A, A is 2,4,6-trichlorobenzoyloxy radical, is obtained by contacting an acid  $\Omega$ -CO-OH, as defined according to claim 1, with 2,4,6-trichlorobenzoyl chloride in  
10 presence of one or more O-acylation additives.

15. The process according to anyone of claims 1 to 8, wherein radical A is corresponding to the following formula:



15

16. The process according to claim 15, wherein reagent of formula  $\Omega$ -CO-A is obtained by contacting an acid  $\Omega$ -CO-OH, as defined according to claim 1, with carbonyl-diimidazole in presence of a strong base.

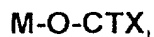
- 20 17. The process according to claim 16, wherein strong base is an alkoxide.

18. The process according to previous claims, wherein the coupling additive is a substituted carbodiimide and/or a basic additive such as tertiary amine for example.

25

19. The process according to claim 18, wherein the substituted carbodiimide is selected from cyclohexylcarbodiimide (DCC), 1,3-diisopropylcarbodiimide (DIC) and chlorhydrate of 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide.

20. The process according to anyone of previous claims, wherein cephalotaxine alcoxide is corresponding to the following formula:



where M and CTX are as defined according to claim 1,

10

is obtained by contacting a cephalotaxine of the following formula:



where CTX is as defined according to claim 1

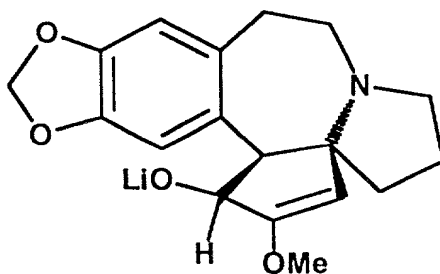
15

with metal himself, an amidure, a metallic hydride or an alkyl-metal.

21. The process according to anyone of claims 1 to 20, wherein M is alkaline metal such as lithium, potassium or sodium.

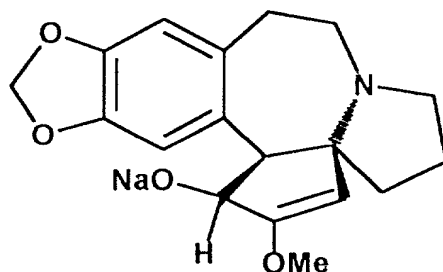
20

22. The lithium alcoxide of cephalotaxine is corresponding to the following formula:

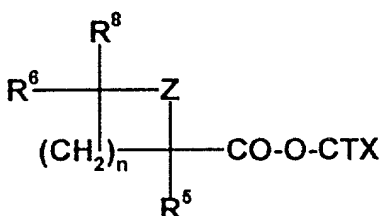


25

23. The sodium alcoxide of cephalotaxine is corresponding to the following formula:



24. A sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:



where

n is included between 0 and 8;

Z is oxygen, nitrogen or sulfur heteroatom;

R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are independently

hydrogen;

hydrocarbon radical, saturated, insaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, heterocycloalkyl, of said radical including or not heteroatom(s);

oxygen ether bearing one of the former radicals;

CTX is as defined according to anyone of claims 1 to 3;

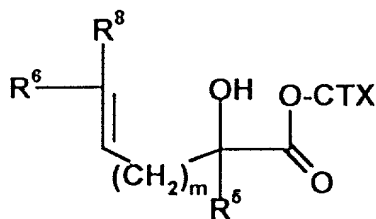
except for compounds where Z is oxygen atom and,

1°) n = 2 or 3, and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OMe or hydroxyl,

1°) n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OMe or hydroxyl;

3°) n = 3 and simultaneously R<sup>6</sup> is hydroxyl, when R<sup>8</sup> is methyl and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical.

25. A sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:



5

where

$m$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined according to claim 1, and CTX is as defined according to anyone of claims 1 to 3,

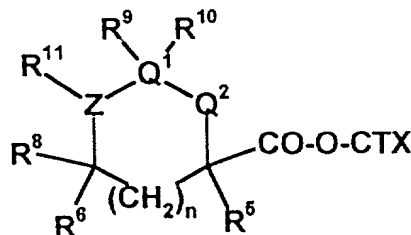
10

Except compound where  $m = 2$ ,  $R^5 = \text{CH}_2\text{CO}_2\text{CH}_3$ ,  $R^6 = R^8 = \text{methyl}$  and CTX is as defined according to claim 3.

15

26. The cephalotaxane according to claim 25, wherein  $R^5$  is  $-\text{CH}_2\text{-CO-O-CH}_3$  radical.

27. A sidechain-bearing cephalotaxane corresponding to the following formula and/or a salt thereof:



20

where  $n$ ,  $Z$ ,  $Q^1$ ,  $Q^2$ ,  $R^5$ ,  $R^6$ ,  $R^8$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  are as defined according to claim 1, and CTX is as defined according to anyone of claims 1 to 3,

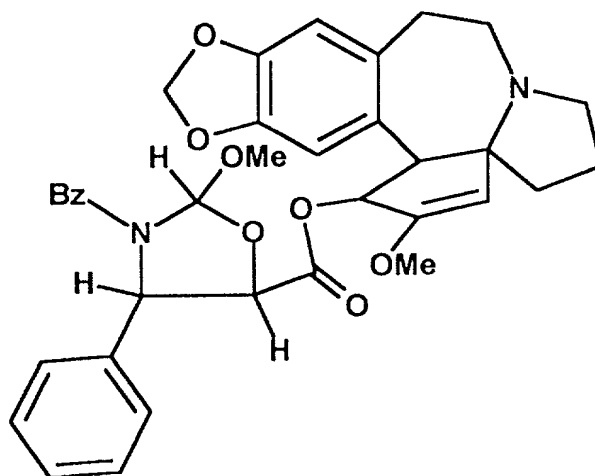
28. The cephalotaxane according to claim 27, wherein  $Q^2$  is oxygen atom.

29. The cephalotaxane according to claim 28, wherein Z is nitrogen atom.

30. The cephalotaxane according to claim 29, wherein  $n = 0$ .

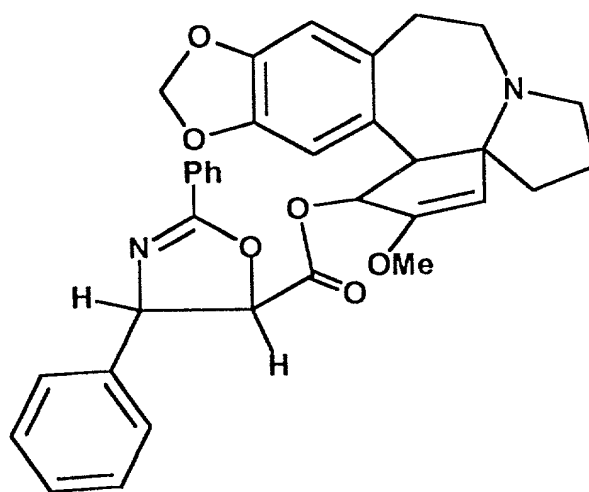
5

31. The sidechain-bearing cephalotaxane corresponding to the following formula:

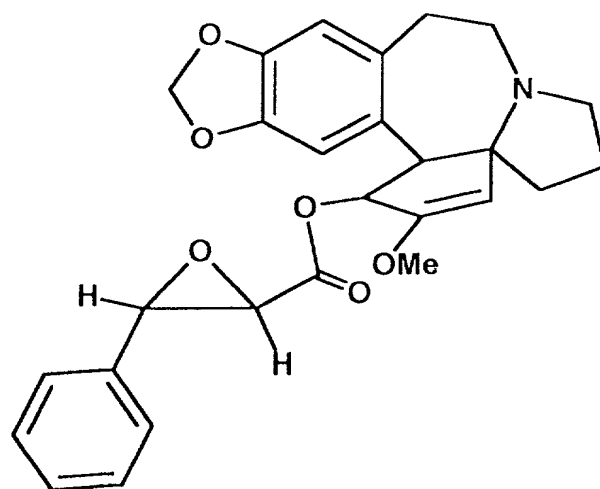


32. The sidechain-bearing cephalotaxane corresponding to the following formula:

10

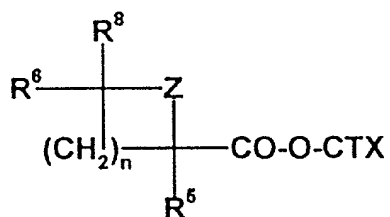


33. The sidechain-bearing cephalotaxane corresponding to the following formula:



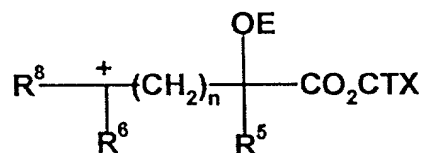
34. The process according to anyone of claims 1 to 21, wherein

- 5 - when the cyclic side-chain of sidechain-bearing cephalotaxane, and/or a salt thereof, of the following formula:



where  $n$ ,  $R^5$ ,  $R^6$ ,  $R^8$ , CTX and Z are as defined according to claim 1;

- 10 the said chain is open with an agent and/or a protonic or not protonic electrophilic radical E in aqueous or not aqueous medium, to provide an intermediate compound of the following formula:

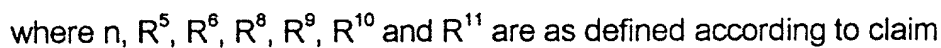


where  $n$ , CTX,  $R^5$ ,  $R^6$  and  $R^8$  are as defined above, E is either hydrogen or the provisionally or definitively fixed electrophilic radical;

15

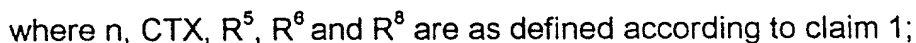
5

- 10



the said chain is open by hydrolysis or carefully solvolysis with possibly presence of activation and/or opening additive;

- 20

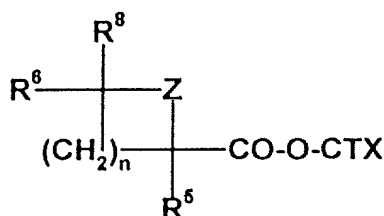


- either an halogen or an heteroatom bearing a hydrogen or a radical R<sup>11</sup> such as defined according to claim 1;

- or an hydrogen, hydrocarbon radical, the said radical bearing or not heteroatom(s), saturated, insaturated or aromatic, linear or ramified and/or cyclic, especially alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, or

heterocycloalkyl.

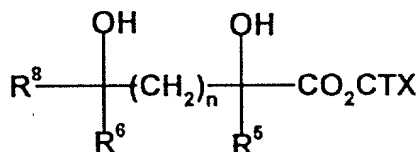
35. The process according to the claim 34 wherein the cyclic side-chain of sidechain-bearing cephalotaxane, and/or a salt thereof, of the following formula:



5

where  $n$ ,  $\text{R}^8$ ,  $\text{R}^6$ ,  $\text{R}^5$ ,  $\text{CTX}$  and  $\text{Z}$  are defined according to the claim 1, the said chain is open by treatment with a solution of hydrobromic acid in acetic acid, in an halogenated solvent, preferably dichloromethane, followed by *in situ* hydrolysis to provide, without isolation of the intermediate, a sidechain-bearing cephalotaxane of the following formula:

10



where  $n$ ,  $\text{CTX}$ ,  $\text{R}^5$ ,  $\text{R}^6$  et  $\text{R}^8$  are defined according to claim 1.

15 36. The process according to anyone of claims 1 to 21 and claim 34, wherein acids are corresponding to the following formula :



where  $\Omega$  radical is as defined according to claim 1;

the said formula equivalent to racemic mixture containing compounds of the formulas (+)- $\Omega$ -CO-OH and (-)- $\Omega$ -CO-OH such as (+)- $\Omega$ -CO-OH represents its dextrogyre enantiomer and (-)- $\Omega$ -CO-OH represent its levogyre enantiomer.

20

were obtained

a) by contacting of said racemic mixture or one of its activated form of the



formula



which is as defined according to claim 1;

5 the said racemic mixture or said activated form generating respectively:

- either an anion corresponding to the formula  $(\Omega\text{-CO-O})^-$ ;
- or a cation corresponding to the formula  $(\Omega\text{-CO})^+$ ;

10 with a pure enantiomeric form of chiral entity, said "resolution agent" symbolized by  $\Delta^*$  (delta stella), having the ability to form:

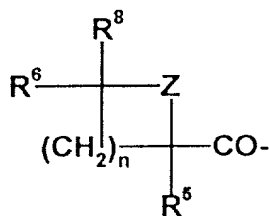
- either a stable combination, by covalent bonding;
- or an easily reversible labil combination, by hydrogen bonding or by hydrophobic interaction;
- 15 - or intermediate lability bonding by electrostatic interaction;

to provide a diastereomeric mixture of  $\Omega\text{-CO-O-}\Delta^*$  and de  $\Omega\text{-CO-}\Delta^*$ ;

20 b) then by physical separation of the mixture of two diastereomers or two complex compounds or more generally of two new entities physically and/or chemically different then obtained;

c) then by regeneration and finally separation of each one of enantiomers of the generic formula  $\Omega^*\text{-CO-OH}$ ,  
 25 where  $\Omega^*$  («oméga stella») represents the generic symbol of the same chiral radical in the either one or the other pure enantiomeric forms corresponding to the following formulas  $(+)\text{-}\Omega\text{-CO-OH}$  and  $(-)\text{-}\Omega\text{-CO-OH}$  which are as defined above.

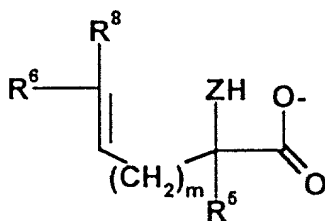
30 37. The process according to claim 36, wherein  $\Omega\text{-CO-}$  is a radical corresponding to the following formula:



where  $n$ ,  $Z$ ,  $R^6$ ,  $R^8$ , and  $R^5$  are as defined according to claim 1.

38. The process according to claim 36, wherein  $\Omega$ -CO- is a radical corresponding to the following formula:

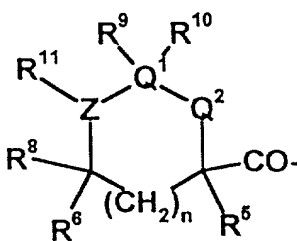
5



where  $m$ ,  $Z$ ,  $R^6$ ,  $R^8$ , and  $R^5$  are as defined according to claim 1.

10

39. The process according to claim 36, wherein  $\Omega$ -CO- is a radical corresponding to the following formula:



15

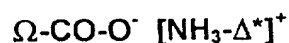
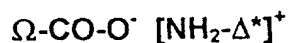
where  $n$ ,  $R^5$ ,  $R^6$ ,  $R^8$ ,  $Z$ ,  $Q^2$ ,  $Q^1$ ,  $R^9$ ,  $R^{10}$  and  $R^{11}$  are as defined according to claim 1.

40. The process according to anyone of claims 36 to 39, wherein the stable combination is represented by an ester of the following formula  $\Omega\text{-CO-O-}\Delta^*$  such as  $\Omega$  and  $\Delta^*$  are as defined according to claim 36, the said stable combination is obtained by contacting acid with a chiral alcohol corresponding to the formula  $\text{HO-}\Delta^*$  such as  $\Delta^*$  is as defined according to claim 36, according to the process of the claim 1.

41. The process according to anyone of claims 36 to 39, wherein the stable combination is represented by an amide corresponding to the either one or the other formulas  $\Omega\text{-CO-NH-}\Delta^*$  or  $\Omega\text{-CO-N-}\Delta^*$  such as  $\Omega$  and  $\Delta^*$  are as defined according to claim 36, the said stable combination is obtained by contacting acid with primary or secondary chiral amine corresponding to formulas  $\text{H}_2\text{N-}\Delta^*$  or  $\text{NN=}\Delta^*$  such as  $\Delta^*$  is as defined according to claim 36, according to the process of the claim 1.

42. The process according to anyone of claims 36 to 39, wherein the stable combination is represented by an thioester of the following formula  $\Omega\text{-CO-S-}\Delta^*$  such as  $\Omega$  and  $\Delta^*$  are as defined according to claim 36, the said stable combination is obtained by contacting acid with a chiral thiol corresponding to the formula  $\text{HS-}\Delta^*$  such as  $\Delta^*$  is as defined according to claim 36, according to the process of the claim 1.

43. The process according to anyone of claims 36 to 39, wherein the ionic combination is represented by a salt just prepared by contacting of acid with a chiral amine corresponding to the either one or the other of the three following formulas:



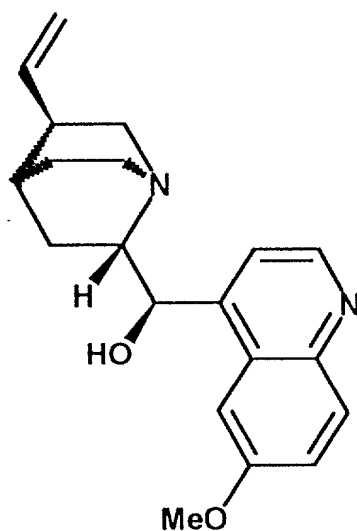
where  $\Omega$  and  $\Delta^*$  are as defined according to claim 36.

44. The process according to anyone of claims 36 to 39, wherein the bringing

into play of a labil bonding based combination is achieved in the form of chromatography with the help of a chiral stationary phase.

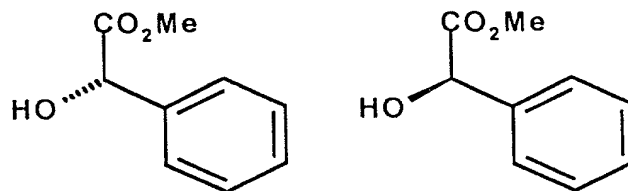
45. The process according to anyone of claims 36 to 39, wherein the bringing  
5 into play of an interatomic or intermolecular labil bonding based combination, within crystalline lattice, is achieved in the form of fractionated crystallization initiated by a chiral precursor.

46. The process according to claim 40, wherein chiral alcohol HO- $\Delta^*$  is (-)-  
10 quinine corresponding to the following formula:

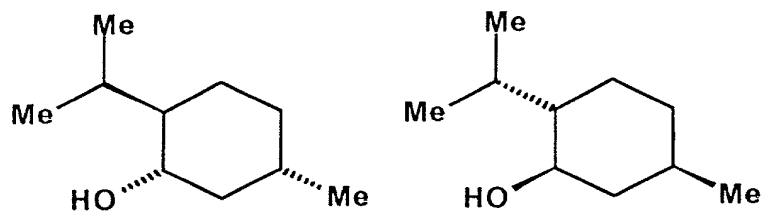


47. The process according to claim 40, wherein chiral alcohol HO- $\Delta^*$  is (-)- or  
(+)-methyl mandelate corresponding to the following formulas:

15

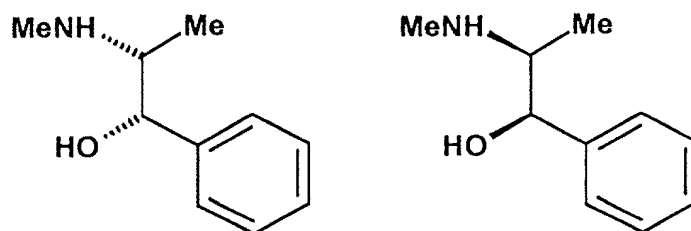


48. The process according to claim 40, wherein chiral alcohol HO- $\Delta^*$  is (-)- or  
(+)-menthol corresponding to the following formulas:

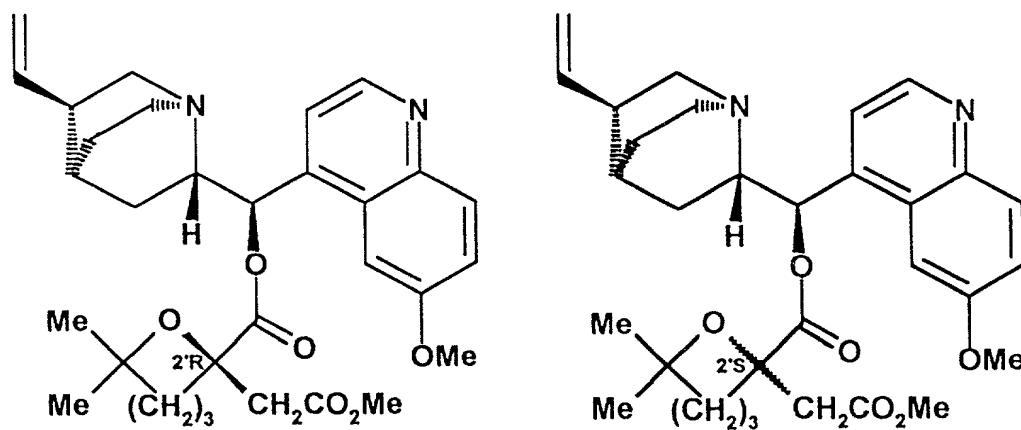


49. The process according to claim 43, wherein chiral amine  $H_2N-\Delta^*$  is (-)- or (+)-ephedrine corresponding to the following formulas:

5

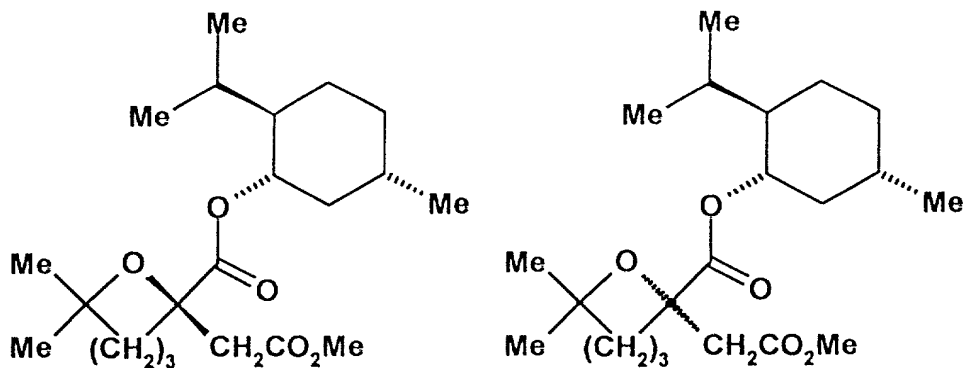


50. The (-)-quinidyl (2'R)-(-)-anhydro-homoharringtonate and the (-)-quinidyl (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:

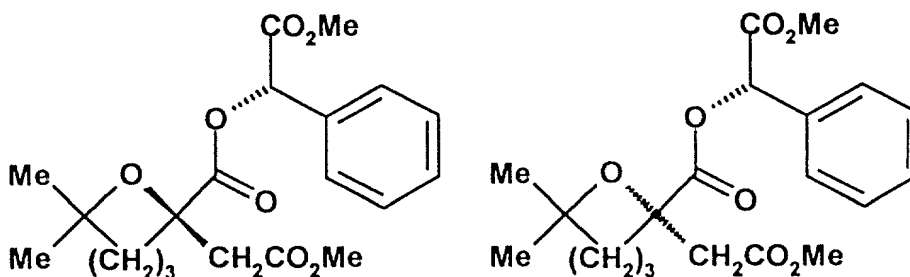


10

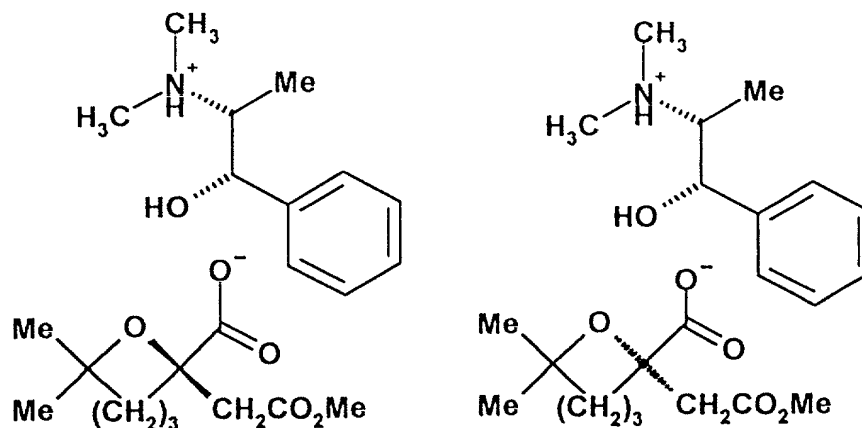
51. The (-)-menthyl (2'R)-(-)-anhydro-homoharringtonate and the (-)-menthyl (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:



52. The (-)-methylmandelyl (2'R)-(-)-anhydro-homoharringtonate and the (-)-methylmandelyl (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:

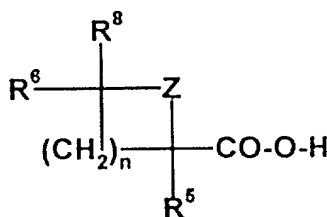


53. The (-)-ephedrinium (2'R)-(-)-anhydro-homoharringtonate and the (-)-ephedrinium (2'S)-(-)-anhydro-homoharringtonate corresponding respectively to the two following formulas:



54. The process according to anyone of claims 1 to 21 and 34 to 49, wherein the carboxylic acid is:

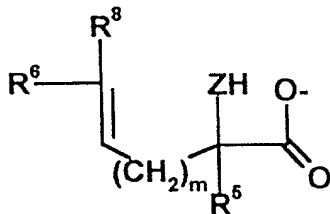
- 5 - either the tertiary heterocycloalkane carboxylic acid corresponding to the following formula:



where  $n$ ,  $Z$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined according to claim 1.

- the said acid is obtained by treatment in aprotic or protic solvent, eventually in the presence of cyclization additive and/or dehydrating agent, the said treatment eventually supported with physical carrying of the water formed.

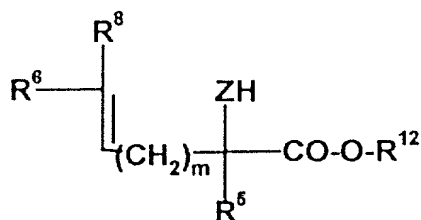
- or open tertiary ethylenic acid corresponding to the following formula:



15

where  $m$ ,  $Z$ ,  $R^5$ ,  $R^6$  and  $R^8$  are as defined according to claim 1.

- or open tertiary ethylenic acid corresponding to the following formula:



where m is included between 1 and 8, Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1, R<sup>12</sup>, is not a CTX radical according to claim 1, represents R<sup>5</sup> and/or a protective group of acids and/or a chiral group; then R<sup>12</sup> is removed later, either just by saponification, or by hydrogenolysis, or more generally by the method of the state of art to remove protective groups of acids.

55. The process according to claim 54, wherein in the absence of cyclization additive, the reaction of cyclization just take place by heating.

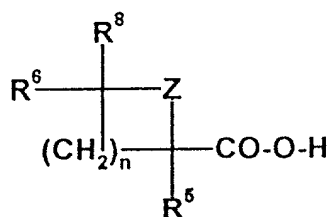
56. The process according to claim 54, wherein the cyclization additive is a protic acid or an aprotic acid, included in immobilized form.

57. The process according to claim 54, wherein the acid is sulfonic acid or formic acid.

58. The process according to anyone of claims 54 to 57, wherein Z is an oxygen atom.

59. The tertiary heterocycloalcane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:





where n is included between 1 and 8, Z, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1, and R<sup>5</sup> is not hydrogen;

except for compounds where Z is oxygen atom and,

5

1°) n = 0 and R<sup>5</sup> is not -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical;

2°) n = 0 and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical, and R<sup>6</sup> = R<sup>8</sup> = methyl or -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical;

10

3°) n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl, and R<sup>5</sup> = OMe or hydroxyl ;

4°) n = 2 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl, and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>H or -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical or methyl;

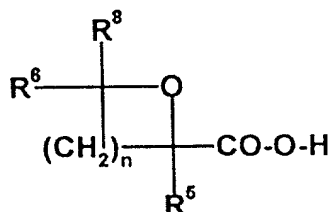
5°) n = 3 and simultaneously R<sup>6</sup> is hydroxyl, and R<sup>8</sup> is methyl, and R<sup>5</sup> is -CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub> radical;

15

6°) n = 3 and simultaneously R<sup>6</sup> = R<sup>8</sup> = methyl and R<sup>5</sup> = OH or methyl or ethyl.

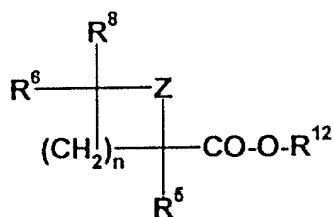
60. The tertiary oxacycloalcane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition,

20 corresponding to the following formula:



where n is included between 0 and 8, R<sup>5</sup>, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 59, but are not hydrogen simultaneously.

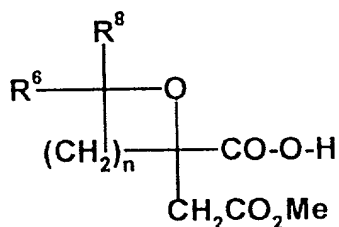
61. The tertiary heterocycloalcanecarboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



5

where  $n$  is included between 0 and 8,  $\text{Z}$ ,  $\text{R}^5$ ,  $\text{R}^6$  and  $\text{R}^8$  are as defined according to claim 59, and  $\text{R}^5$  is not hydrogen, and  $\text{R}^{12}$  is defined according to claim 54.

- 10 62. The tertiary oxacycloalcanecarboxylic hemiester, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

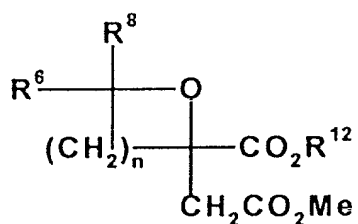


15

where  $n$  is included between 0 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined according to claim 59.

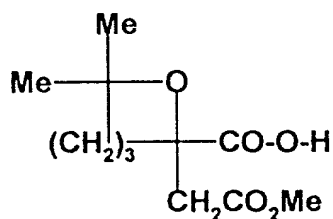
63. The tertiary oxacycloalcanecarboxylic hemiester, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

20



where n is included between 0 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined according to claim 59,  $\text{R}^{12}$  is defined according to claim 54.

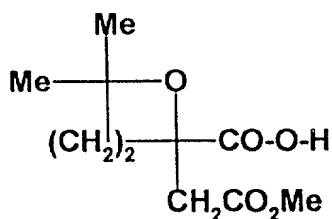
64. The tertiary oxacycloalcanecarboxylic hemiester or anhydro-homoharringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



10

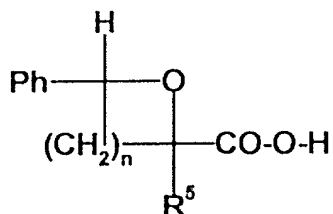
65. The tertiary oxacycloalcanecarboxylic hemiester or anhydro-harringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

15



66. The tertiary oxacycloalcanecarboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition,

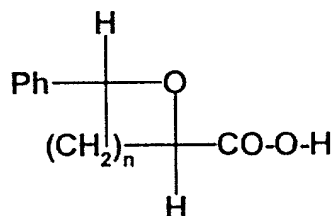
corresponding to the following formula:



where n is included between 0 and 8, R<sup>5</sup> is as defined according to claim 59.

5

67. The tertiary oxacycloalcanecarboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

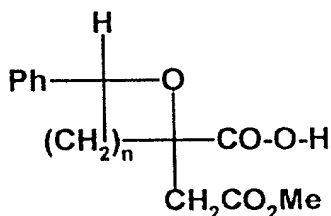


10

where n is included between 1 and 8.

68. The tertiary oxacycloalcanecarboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

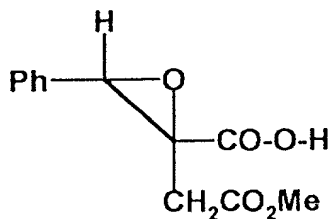
15



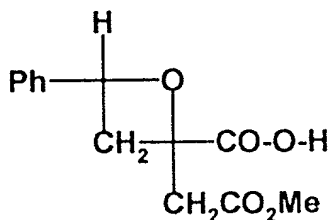
where n is included between 0 and 8.

69. The tertiary oxacycloalcanecarboxylic acid or oxanhydroneoharringtonic

acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

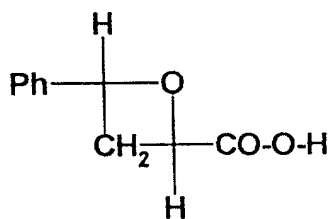


- 5 70. The tertiary oxacycloalkane carboxylic acid or oxanhydro-neohomoharringtonic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

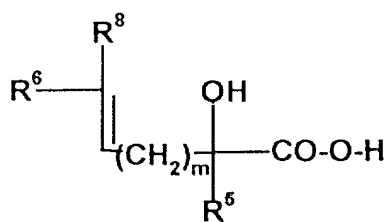


10

71. The tertiary oxacycloalkane carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

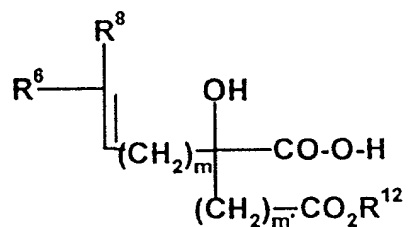


- 15 72. The tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



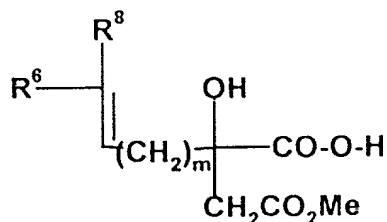
where m is included between 1 and 8, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1, but are not hydrogen simultaneously, and R<sup>5</sup> is not hydrogen or heteroatom.

73. The tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



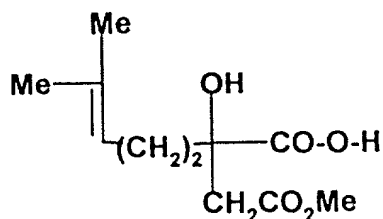
where m is included between 1 and 8, m' is included between 1 to 8, R<sup>6</sup> and R<sup>8</sup> are as defined according to claim 1 and R<sup>12</sup> is defined according to claim 54.

74. The tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:

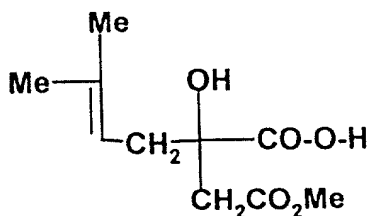


where m is included between 1 and 8,  $\text{R}^6$  and  $\text{R}^8$  are as defined according to claim 1 but are not hydrogen.

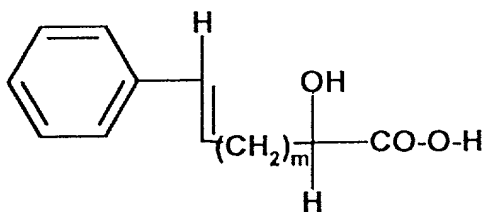
- 5 75. The tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



- 10 76. The tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



- 15 77. The tertiary alkene carboxylic acid, included its salts and each one of its pure enantiomeric forms or in racemic mixture or in variable composition, corresponding to the following formula:



where m is included between 1 to 8.

78. The compound according to claim 77, where m = 1.

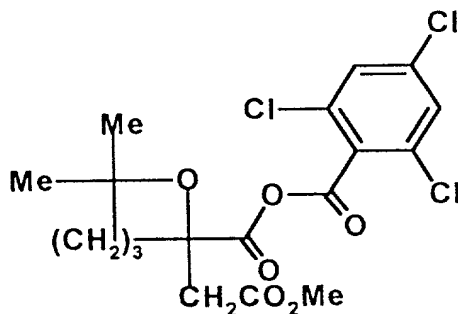
5

79. The anhydrides of acid according to anyone of claims 58 to 70, of the general formula  $\Omega$ -CO-O-CO- $\Omega$  where  $\Omega$  is as defined according to claim 1.

80. The mixed anhydrides of acid according to anyone of claims 58 to 70, of the general formula  $\Omega$ -CO-A where A is as defined according to anyone of claims 12, 13 or 15.

10

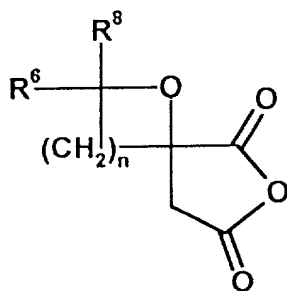
81. The mixed anhydride corresponding to the following formula:



82. The acid chlorides according to anyone of claims 58 to 70, corresponding to the general formula  $\Omega$ -CO-X, where X is halogen.

15

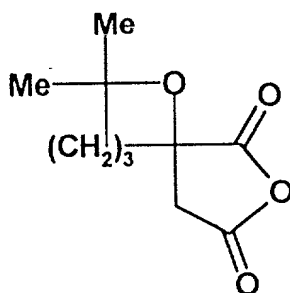




83. The cyclic anhydrides corresponding to the following formula:

5 where  $n$ ,  $R^6$  and  $R^8$  are as defined according to claim 1.

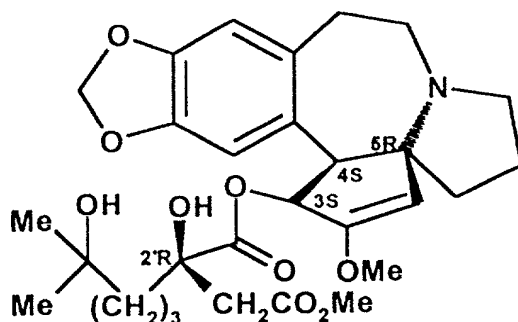
84. The cyclic anhydride corresponding to the following formula:



85. The process according to anyone of claims 1 to 21 and 34 to 49 where the  
 10 sidechain-bearing cephalotaxane was purified like a salt by chromatography  
 using a reversed-phase like stationary phase, and a mobile phase without  
 organic solvent like a solution adjusted to a pH 2 to 4.5 with a buffer prepared  
 with an acid and an alkaline or ammonium salt and one or several additive like  
 attenuator of silanol effect, the said cephalotaxine salt was generated from  
 15 mineral acid under the form of chlorohydrate, sulfate, phosphate, nitrate,  
 perchlorate, or from organic acid under the form of tartrate, malate, citrate or  
 lactate.

86. The process according to anyone of claims 1 to 21 and 34 to 49 comprising a step of chromatographic purification of a natural or semi-synthetic or synthetic homoharringtonine as a pharmaceutical use corresponding to the following formula:

5

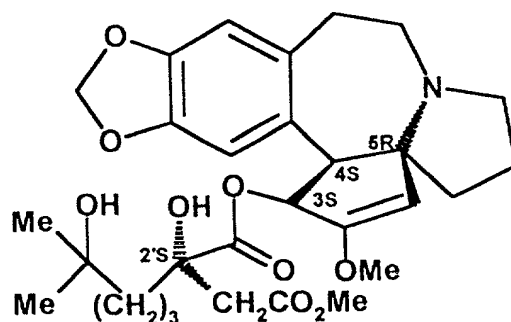


to remove the undesired related impurity named 2'-épi-homoharringtonine resulting:

10

a) either from a semi-synthetic process with introduction of a synthetic homoharringtonic acid of inadequate enantiomeric purity, the generated impurity showing the absolute configuration corresponding to the following formula:

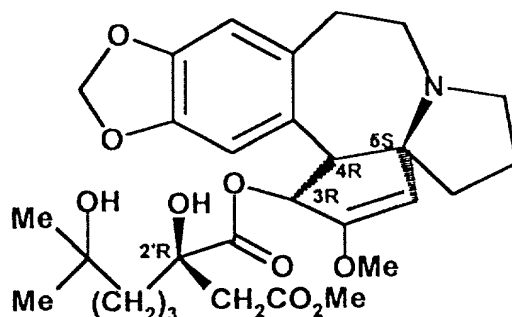
15



b) or from the biosynthetic process in the plant, where a cephalotaxine with inadequate enantiomeric purity was introduced, or in the form of artefact by partial racemization of the cephalotaxine moiety, the generated impurity showing strictly identical chromatographic properties with a non-chiral system,

20

with an absolute configuration opposite to the one above (enantiomer) and corresponding to the following formula:



5 especially making use of one of the following chromatographic systems:

A) Stationary phase:

alkyl- or phenyl- or alkylphenyl- ou phenylalkyl-silane, preferably *n*-octadecylsilane,

B) Mobile phase:

10 water-tetrahydrofurane, water-methanol, water-acetonitrile or buffer pH 2 to 6.5 in replacement of water, or all other mobile phase with equivalent selectivity,

87. The process of purification and chromatographic control according to the  
 15 claim 86 of a natural or semi-synthetic or synthetic homoharringtonine, allowing to offset the double insufficiency of enantiomeric purity of the half-precursors, both on the sidechain precursor (said homoharringtonic acid) and cephalotaxine, the two said-precursors are each independently generated by total synthesis or semi-synthetic process or natural process within of the plant  
 20 (biosynthesis), in fact the withdrawal of the non natural enantiomer of homoharrintonine showing an opposite absolute configuration, by using a chiral stationary phase with preparative scale.

**ABSTRACT**

The present invention concerns a new general process for asymmetric hemisynthesis of harringtonines and their analogs, that are alkaloids used in chemotherapy. This process comprises direct esterification of a natural cephalotaxine with an acylating compound constituted of a side chain precursor which backbone and functionalization are entirely preformed.

09270006 031699



FIGURE 1



COMBINED DECLARATION AND POWER OF ATTORNEY  
FOR UTILITY PATENT APPLICATION

Attorney's Docket No.

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

Novel cephalotaxane derivatives and process for their preparation

the specification of which

(check one)



is attached hereto;



was filed on \_\_\_\_\_ as

International Application No.

and was amended on \_\_\_\_\_;  
(if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

<b>COMBINED DECLARATION AND POWER OF ATTORNEY</b>	Attorney's Docket No. 017753-077
---	-------------------------------------

COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
FRANCE	98 03492	30.03.1998	YES <u>X</u> NO <u>XXX</u>
			YES_ NO_

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

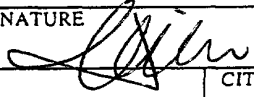
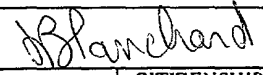
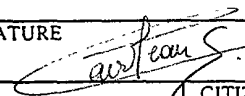
William L. Mathis	17,337	Ralph L. Freeland, Jr.	16,110	William C. Rowland	30,888
Peter H. Smolka	15,913	Robert G. Mukai	28,531	T. Gene Dillahunt	25,423
Robert S. Swecker	19,885	George A. Hovanec, Jr.	28,223	Anthony W. Shaw	30,104
Platon N. Mandros	22,124	James A. LaBarre	28,632	Patrick C. Keane	32,858
Benton S. Duffert, Jr.	22,030	E. Joseph Gess	28,510	Bruce J. Boggs, Jr.	32,344
Joseph R. Magnone	24,239	R. Danny Huntington	27,903	William H. Benz	25,952
Norman H. Stepno	22,716	Eric H. Weisblatt	30,505	Peter K. Skiff	31,917
Ronald L. Grudziecki	24,970	James W. Peterson	26,057	Richard J. McGrath	29,195
Frederick G. Michaud, Jr.	26,003	Teresa Stanek Rea	30,427	Matthew L. Schneider	32,814
Alan E. Kopecki	25,813	Robert E. Krebs	25,885	Michael G. Savage	32,596
Regis E. Sluter	26,999	Robert M. Schulman	31,196	Gerald F. Swiss	30,113
Samuel C. Miller, III	27,360				

and: \_\_\_\_\_

Address all correspondence to: **Norman H. Stepno**  
**Burns, Doane, Swecker & Mathis, LLP**  
 P.O. Box 1404  
 Alexandria, Virginia 22313-1404


Address all telephone calls to: Norman H. Stepno at (703) 836-6620.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF SOLE OR FIRST INVENTOR ROBIN Jean-Pierre	SIGNATURE 	DATE Feb. 3, 1999
RESIDENCE 28, rue Sainte Croix 72000 LE MANS - FRANCE		CITIZENSHIP FR
POST OFFICE ADDRESS the same as residence		
FULL NAME OF SECOND JOINT INVENTOR, IF ANY BLANCHARD Julie	SIGNATURE 	DATE FEB. 3, 1999
RESIDENCE 17, rue de l'Alma 72000 LE MANS - FRANCE		CITIZENSHIP FR
POST OFFICE ADDRESS the same as residence		
FULL NAME OF THIRD JOINT INVENTOR, IF ANY CAVOLEAU Sylvie	SIGNATURE 	DATE Feb. 3, 1999
RESIDENCE 52, rue Chèvre 49000 ANGERS - FRANCE		CITIZENSHIP FR
POST OFFICE ADDRESS the same as residence		



Full name of 4th joint inventor: CHAUVIAT Ludovic

Fourth Inventor's signature: 

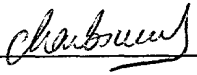
Date: 03/02/1999

City and Country of Residence: 4, rue Bossuet - 72000 LE MANS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 5th joint inventor: CHARBONNEL Sandra

Fifth Inventor's signature: 

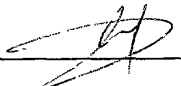
Date: 03/02/1999

City and Country of Residence: 23, avenue Vauban - 49000 ANGERS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 6th joint inventor: DHAL Robert

Sixth Inventor's signature: 


Date: 03/02/1999

City and Country of Residence: Le Champ du Dragon - 12, rue des Bois -  
72700 PRUILLE LE CHETIF - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 7th joint inventor: DUJARDIN Gilles

Seventh Inventor's signature: 


Date: 03/02/1999

City and Country of Residence: 5, rue de l'Ormeau - 72700 ROUILLON - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 8th joint inventor: FOURNIER Florence

Eighth Inventor's signature: 

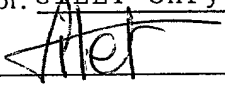
Date: 3/02/99

Apt A 504 - 4, rue Paul Courboulay  
City and Country of Residence: 72000 LE MANS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 9th joint inventor: GILET Chrystelle

9th Inventor's signature: 


Date: 3/02/99

City and Country of Residence: 97, avenue Bollée - 72000 LE MANS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 10th joint inventor: GIRODIER Laurent

Tenth Inventor's signature: 

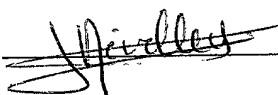
Date: 03/02/99

City and Country of Residence: 215, avenue Rubillard - 72000 LE MANS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 11th joint inventor: MEVELEC Laurence

11th Inventor's signature: 

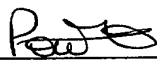
Date: 03/02/99

City and Country of Residence: 17, rue des Remparts - 27400 LOUVIER - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 12th joint inventor: POUTOT Sandrine

12th Inventor's signature: 

Date: 03/02/1999

City and Country of Residence: 126, rue de Funay - 72000 LE MANS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of 13th joint inventor: ROUAUD Sylvie

13th Inventor's signature: 

Date: 3/2/1999

City and Country of Residence: 213, avenue Rubillard - 72000 LE MANS - FRANCE

Citizenship: French

Post Office Address: The same as residence

Full name of joint inventor: \_\_\_\_\_

Inventor's signature: \_\_\_\_\_

Date: \_\_\_\_\_

City and Country of Residence: \_\_\_\_\_

Citizenship: \_\_\_\_\_

Post Office Address: \_\_\_\_\_

Full name of joint inventor: \_\_\_\_\_

Inventor's signature: \_\_\_\_\_

Date: \_\_\_\_\_

City and Country of Residence: \_\_\_\_\_

Citizenship: \_\_\_\_\_

Post Office Address: \_\_\_\_\_